20th EUCARPIA General Congress

Abstracts

29 Aug – 1 Sep 2016
ETH Zurich, Switzerland

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Plant Breeding: the Art of Bringing Science to Life

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Roland Kölliker
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Technical editors
Anna Dalbosco
Paolo Demaria

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Preface

Plant breeding plays a pivotal role in securing the production of healthy feed and food while at the same time optimizing resource utilization and minimizing environmental impacts. The growing demand through a rapidly increasing world population further accentuates the need for improved cultivars. Progress in plant breeding has always been achieved through a close interplay of the art of selecting suitable germplasm and science to develop the knowledge and tools to make the right breeding decisions. The development of new tools and the rapid technological development of plant genomics, bioinformatics and phenomics, to name just a few, have added a new dimension to combining art and science in plant breeding. A clear understanding of the requirements for the plant cultivar of the future is necessary for the targeted application of these tools and techniques. At the same time, breeding objectives are constantly changing as a result of climate change, a scarcity of natural resources and the necessity to sustain biodiversity. The various disciplines must collaborate to achieve the common goal of developing new cultivars, thus translating these scientific advances into action. Thus, when given the opportunity to organize the 20th Eucarpia General Congress, we felt it appropriate to put it under the theme "Plant breeding: the art of bringing science to life" and to use it as a platform to explore how technical advances and growing genomic knowledge are transforming plant breeding and how these advances can be integrated into the development of novel, improved plant material.

We were overwhelmed by the positive response to our proposal, which enabled us to create an exciting scientific program covering many aspects of plant breeding and plant science research. With more than 420 registered participants, this conference brings together breeders, gene bank curators, plant scientists and molecular biologists from 48 countries and five continents. This book contains the abstracts of the twelve keynote presentations and more than 320 offered oral or poster presentations by renowned scientists, organized in twelve plenary and thirteen parallel, crop specific sessions. Sessions span a broad range of topics including genomics, stress tolerance, secondary metabolites, phenomics, genetic resources and plant - microbe interactions. The large number of diverse scientific presentations, together with the social events and the mid conference excursions, will with no doubt sparkle lively discussions on the interplay of plant breeding and plant science and hopefully result in many novel fruitful collaborations.

Finally, we would like to thank all the people who made this event possible. The colleagues of ETH Zürich for hosting us in a stimulating environment, the program committee for putting together an excellent program, the extended scientific committee for assisting with selecting presentations and reviewing abstracts and the local organizing committee for their tremendous effort in organizing every detail.

We wish all the participants an inspiring conference and welcome you all to Zurich.

Zurich, Switzerland

Beat Boller
Roland Kölliker

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Parallel flash and poster presentations: Fodder crops and Maize

Opening session

Plant breeding: Past, present and future

John E. Bradshaw

Retired Plant Breeder and Geneticist, Scotland

johnbradshaw949@btinternet.com

I had a 34 year career from 1975 to 2009 as a plant breeder and geneticist at what is now the James Hutton Institute in Dundee, Scotland. Plant breeders starting a 34 year career in 2016 will be finishing in 2050, by when the global human population will have risen to 9.0 billion, from the 7.0 billion reached in 2011. Farmers can try to produce the extra food that will be required by bringing more land into cultivation and by increasing yield per hectare, as has happened in the former fenland of Eastern England since 1600. The UN predicted increase in cultivated land is only 10 per cent, so a large increase will be required in current global yields estimated at 55 per cent of attainable yields. Plant breeders can help farmers increase food production by breeding new cultivars better adapted to their chosen farming systems, but these must be capable of providing the necessary plant inputs for the required levels of crop production in 2050. The crops grown and their growing seasons will be determined by temperature and water supply, the two factors most likely to be adversely affected by climate change. As explained in my book (Bradshaw, 2016), until 200 years ago the farmers themselves were also the plant breeders, or rather the selectors. The outcome of plant domestications, extensive crop dispersions and farmer selection, was thousands of locally adapted landraces of cultivated plants which could be grouped into geographical races and ecotypes. During the 20th century numerous landraces were replaced by relatively few high yielding cultivars, and land degradation and changes in use endangered the habitats of many of their wild relatives. Hence *in situ* and *ex situ* conservation, evaluation and use of plant genetic resources is vital for the success of future plant breeding. Deliberate hybridization in plant breeding started at the beginning of the 19th century. The prerequisite was an understanding of sexual reproduction in flowering plants which was provided by botanists and microscopists from 1682 to 1847. Extensive experimentation in plant hybridization was done from the mid 18th to mid 19th century, first by naturalists, then also by breeders. By 1900 plant breeding was established both as a discipline and as a profession. The development of scientific plant breeding from the beginning of the 20th century was based on understanding the mechanism of inheritance and the mating systems of crop plants. The latter, together with the mode of reproduction (sexual or asexual), determined the types of genetically uniform, high yielding cultivars that have been bred from genetically heterogeneous landraces: inbred line (wheat) and hybrid (rice) cultivars for inbreeding species, hybrid (maize) cultivars for outbreeding species, and clonal (potato) cultivars for vegetatively propagated species. Future progress in crop improvement will come from three complementary approaches: use of sexual reproduction in further conventional breeding, base broadening and introgression; mutation breeding; and genetically modified crops. Breeders will benefit from increased genetic knowledge and combine it with technological advances to aid the discovery of desirable genes (alleles) and to make breeding faster, more efficient and more effective at achieving desired goals. But breeders will still need to apply appropriate breeding methods to the right germplasm for the right objectives. The latter will depend upon answering the big questions about the most appropriate farming systems and most appropriate uses of crops in 2050, and there does need to be a sense of urgency and an appreciation of the scale of breeding required.

Bradshaw JE (2016) Plant Breeding: Past, Present and Future. Springer, New York, 693p.

Plenary session 1a:
Genomics and bioinformatics I

Big genomes, big data, big progress? What will be the impact of reference genome sequences in barley, wheat and rye?

Nils Stein[a]

[a] *Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany*

stein@ipk-gatersleben.de

Barley, wheat and rye are closely related Poaceae species belonging to the tribe Triticeae. They all represent crop species of global importance; wheat and barley are two of the three most important crops of Europe. Despite their economic importance, the three species are lagging behind many other plant / crop species in respect to access to their genomes and gene complements – basic resources required for establishing genomics-based breeding strategies, which are seen as important component of the tool box that is needed to tackle future global challenges to feed a growing world population under changing environment and increasingly limited water, arable land and fertilizer resources.

Until the availability of *Next Generation Sequencing* technology, the genomes of wheat (17 Gbp), barley (5 Gbp) and rye (7 Gbp) were just too expensive to be sequenced. But also the structure of their genomes; composed of only 2 % of protein coding sequences and of 80-90 % of repetitive DNA – sequences that are present as identical or very similar copies hundreds or thousands of times in the genome, was too complex for many strategies of whole genome sequencing. However, due to the perseverance of international wheat and barley genome sequencing consortia (IWGSC, www.wheatgenome.org; IBSC, barleygenome.org), reference sequences of both barley and wheat are soon publicly available.

The production of reference sequences of wheat and barley required the generation of very large sequence datasets. Also all applications taking advantage of these genomic datasets are likely to generate (if not multiply) even larger datasets. The efficient utilization of genomic data especially in large genome crop species requires the ability of handling and storing BIG DATA; thus we see today a serious shift of required capabilities for the young generation of scientists that enter the area of Life Sciences / Agricultural Sciences and Breeding.

Access to reference sequences of wheat and barley is removing major limitations for research and application. The availability of reference sequences for both crop species is already changing our approaches of how genetic factors underlying crop performance are being isolated. However, the international research community has to proof now that the wheat and barley genome sequences allow us to establish genomics-based crop improvement strategies to help closing the yield gap.

Genomic assisted selection and classical plant breeding – synergy or competition?

Yonatan Elkind

Institute of Plant Science and Genetics in Agriculture, The Hebrew University of Jerusalem, Israel

Yonatan.Elkind@mail.huji.ac.il

The dramatic reduction in the cost of genotyping (especially of SNPs), has made readily available a large number of markers for many individuals of crop plants. Such information together with phenotyping, advanced analysis methods and models has generated an explosion of genetic information regarding the association between the markers and genes controlling quantitative traits (QTLs). Our understanding of the genetic control of those traits, (which are referred to also as complex traits), has allowed us to gradually decipher the random genetic variation into specific QTLs. But still in most cases the DNA polymorphisms causing the genetic effect have not been identified and we identify those genes by association – linkage to markers. Furthermore, most models assume the lack of interaction between genes and have limited ability in handling genotype by environment interactions. The dramatic growth in data and knowledge of quantitative trait is very impressive, but it still explains only a portion of the genetic variation.

DNA markers have been used very efficiently in plant breeding programs to select for single genes such as disease resistance genes or major genes affecting development. Using more data and advanced models in Marker Assisted Selection (MAS) and more recently Genomic Assisted Selection (GAS), have been adopted by commercial breeding projects and resulted in dramatic improvements and achievements. Although no one doubts those achievements, it is argued that the cost of MAS should be compared to the cost of classical plant breeding based on phenotypic selection. Most probably, allocation of similar resources to highly qualified teams of plant breeders will result in dramatic enhancement of the achievements of classical breeding. The field of plant breeding has enjoyed a much higher level of investment in the development of GAS. The justification for this investment was that it would lead to more rapid improvement in plant improved genotypes. The advancements achieved so far are not in question, but it is claimed that this is a result of greater financial investment, high levels of human resources and larger than usual genetic variation. Instead of advocating GAS as a modern replacement to conventional plant breeding, it is proposed that the High-Throughput Genotyping and Genome Wide Association Studies - GWAs (especially for quantitative traits) should be applied as an important tool for the identification of the genetic basis of the various traits. This knowledge should be integrated in better supported plant breeding projects and assist in selection whenever it is more efficient.

The consistent contribution of plant breeding to yield increases and quality improvements in crop plants resulted from the ability of breeders to apply limited genetic knowledge and overcome huge gaps in knowledge by phenotypic selection of superior and in many cases unexpected genotypes. Despite the dramatic increase in knowledge, it is still limited, hence, synergy between classical plant breeding and genomic knowledge is called for. It will lead to the desired optimization of investment in plant breeding and maximize achievements.

Transcriptomics to understand host adaptation in cereals powdery mildews

Coraline R. Praz[a], Salim Bourras[a], Fabrizio Menardo[a] , Thomas Wicker[a] and Beat Keller[a]

[a] *Department of Plant and Microbial Biology, University of Zürich, Switzerland*

coraline.praz@botinst.uzh.ch

Powdery mildew are agronomically important and widespread fungal pathogens of crop plants. *Blumeria graminis* (*B.g.*) the causal agent of cereal powdery mildews, is a biotrophic fungus. *Blumeria graminis* is divided in several *formae speciales* (f.sp) depending on the host the pathogen grows on. *B.g.* f.sp. *tritici* and *B.g.* f.sp. *secalis* are wheat and rye powdery mildew respectively. Triticale is an artificial hybrid of wheat and rye developed to combine the yield potential and grain quality of wheat and the disease and environmental tolerance of rye. It was initially resistant to powdery mildew but in 2001 the pathogen was observed on triticale. It became since then a major triticale disease in Europe. It was recently shown that the evolution of the pathogens mirrors the evolution of the hosts: triticale powdery mildew (*B.*g. f.sp. *triticale)* is a hybrid between wheat powdery mildew and rye powdery mildew. At the genomic level, *B.g. triticale* is very similar to *B.g. tritici* and can infect both wheat and triticale.

In this work, we want to identify genes responsible for host adaptation in different cereal powdery mildews and more especially genes responsible for the gain of virulence of powdery mildew on triticale. For this we used a RNASeq approach to study compatible interactions during formation of haustorium, a feeding structure mediating successful pathogen infection and disease progression (two days post infection). Sequencing data were generated from i) wheat leaves infected with three different *B.g. tritici* isolates, ii) rye leaves infected with two *B.g. secalis* isolates and iii) triticale leaves infected with two *B.g. triticale* isolates. We hypothesized that genes that are differentially between *formae speciales* at this stage of the infection are important for the adaptation to a particular host.

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Menardo F., Praz, C., Wyder, S., Ben-David, R., Bourras, S. A., Matsumae, H., McNally, K. E., Parlange, F., Riba, A., Roffler, S., Schaefer, L. K., Shimizu, K. K., Valenti, L., Zbinden, H., Wicker, T., and Keller, B. Hybridization of powdery mildew strains gives raise to pathogens on novel agricultural crop species. Nat. Genet., (2016).

Wicker, T., et al. The wheat powdery mildew genome shows the unique evolution of an obligate biotroph. Nat. Genet., (2013).

Wisdom of crowds: pooled sequencing identifies genomic regions associated with disease resistance in ryegrass

Verena Knorst[a], Stephen Byrne[b], Torben Asp[c], Bruno Studer[d], Franco Widmer[a] and Roland Kölliker[a]

[a] *Institute for Sustainability Sciences INH, Agroscope, Switzerland*
[b] *Crops, Environment and Land Use Programme, Teagasc, Ireland*
[c] *Molecular Biology and Genetics, Aarhus University, Denmark*
[d] *Forage Crop Genetics, ETH Zurich, Switzerland*

verena.knorst@agroscope.admin.ch

Italian ryegrass (*Lolium multiflorum*) is an important fodder grass of temperate regions. Harvest may be severely reduced due to infection with the wilt causing bacterium *Xanthomonas translucens* pv. *graminis* (*Xtg*). Genomic markers linked to disease resistance against this pathogen may facilitate exploitation of the naturally occurring resistance or introduction of novel resistance alleles in breeding programs. In this project, a major quantitative trait locus (QTL) previously identified in the biparental mapping population ART-*Xtg* (Studer, 2006), was fine-mapped to a region of 1.5cM of linkage group (Lg) 4 and the nearest marker explained up to 40% of the resistance against *Xtg*. DNA of the two parental genotypes of the mapping population was sequenced using the Illumina HiSeq2000 platform. Both genomes were assembled with the ABySS software and the assembly of the resistant parent was chosen as a reference for further analysis. $18x10^6$ heterozygous single nucleotide polymorphisms (SNPs) were called between the parents. To identify the most resistant and most susceptible offspring, disease scores from different infection experiments were used. DNA of the most resistant and most susceptible quartile of the offspring (57 and 50 individuals, respectively) were pooled and sequenced using the same method as for the parents. These reads were aligned to the reference and 67% ($12x10^6$) of the parental SNPs were covered by reads from the pools. The Fixation index (F_{ST}) was calculated for each SNP to identify SNPs correlated with the reads from the resistant pool and SNPs with F_{ST} values ≥ 0.9 were considered as correlated with resistance. The sequences of 140 scaffolds containing such SNPs were compared with the sequences of the scaffolds of *Lolium perenne* (Byrne, 2015) and located on the *Lolium perenne* linkage map with the use of the GenomeZipper (Pfeifer, 2013). Scaffolds mapping only to Lg4 or mapping to Lg4 and showing homologies to genes correlated with resistance were chosen as candidate scaffolds. Several candidate scaffolds showed homologies to serine/threonine kinases. Mapping of the first two scaffolds using HRM and direct sequencing located these markers in the QTL region of Lg4. Additional SNPs will be mapped using the KASP assay. This study indicates that sequencing of pools of individuals differing strongly in one trait can be used to identify genomic regions correlated with the trait and due to the sequencing based approach marker are directly available for usage in breeding programs.

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Pfeifer M. et al., 2013, The Perennial Ryegrass GenomeZipper: targeted use of Genome resources for comparative grass genomics, Plant Physiology 161(2):571-82

BARLEX – the barley draft genome explorer

Christian Colmsee[a], Sebastian Beier[a], Axel Himmelbach[a], Thomas Schmutzer[a], Nils Stein[a], Uwe Scholz[a] and **Martin Mascher**[a]

[a] *Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Germany*

mascher@ipk-gatersleben.de

The progress in sequencing technology has facilitated the development of advanced genomics infrastructures of species with large and complex genomes. Visualizing these multi-layered frameworks requires the integration of diverse data from disparate sources. Existing genome browsers are not well adapted to this task as they expect all genomic features to be in the correct linear order. We developed the barley genome explorer BARLEX, as a central repository for the genomic resources of barley (*Hordeum vulgare* L.). BARLEX is centered on the physical map of barley and links it to an annotated whole-genome shotgun assembly and dense genetic maps. A web-based interface provides access to all information and sequence data associated with shotgun assemblies, physical contigs and annotated genes. We implemented a graph-based visualization strategy to show overlaps between adjacent bacterial artificial chromosomes (BACs) based on their sequences. BARLEX is designed as an expandable one-stop shop to accommodate the accruing sequence data of the on-going barley genome sequencing project and provides a toolkit to guide the automated construction of a reference sequence of barley. The design of BARLEX can serve as blueprint for setting up similar browsers for other species such as wheat or rice. BARLEX is publicly accessible at http://barlex.barleysequence.org.

Plenary session 1b:
Genomics and bioinformatics II

Towards genomic selection in an outbreeding crop, perennial ryegrass

Torben Asp

Department of Molecular Biology and Genetics, Aarhus University, Denmark

torben.asp@agrsci.dk

Integrating high-throughput phenotyping technologies in a multi-trait genomic prediction model

Daniela V. Bustos-Korts[ab], Marcos Malosetti[a], Scott C. Chapman[c], Bangyou Zheng[c] and Fred A. van Eeuwijk[a]

[a] *Biometris, Wageningen University and Research Centre, The Netherlands*
[b] *C.T. de Wit Graduate School for Production Ecology & Resource Conservation (PE&RC), The Netherlands.*
[c] *CSIRO Agriculture, Australia.*

daniela.bustoskorts@wur.nl

Prediction of the final state of a complex and composite trait can be improved by adding information on the dynamics of this trait (yield) and its constituent components. Information on dynamics can be obtained from high-throughput phenotyping and can be summarized by parametric or semi-parametric statistical growth models. The parameters of these models are expected to have a larger heritability and provide a better integration of the plant responses to environmental conditions, compared to information from single measurement time points. Therefore, curve parameters can be used as correlated traits in multi-trait genomic prediction models.

Multi trait genomic prediction models can be useful in the following scenarios; 1) prediction of final yield from measurements of yield components at early stages of the growth cycle (selection during the growing season) and 2) improving prediction accuracy of yield by integrating yield and yield components (selection at harvest). The degree of success of both prediction scenarios largely depends on the covariance structure among yield and yield components and on the heritability of each trait.

To evaluate both prediction scenarios, we simulated a F8-RIL population of 700 genotypes segregating for APSIM-Wheat parameters regulating phenology, biomass partitioning and ability to capture environmental resources. The parameter values of the segregating population were used to simulate yield, yield components and phenology during the growth cycle in Australian environments. We consider different experimental and measurement error scenarios and discuss possible extensions to the multi-environment case.

QTL detection in multi-parent population using different types of QTL effects and cross specific residual terms models

Vincent Garin, Marcos Malosetti, Fred van Eeuwijk

Plant Sciences Group/Biometris, Wageningen University, The Netherlands

vincent.garin@wur.nl

Multi-parent populations (MPPs) are a useful resource to investigate biological questions in plants. MPPs are composed of genotypes coming from several connected crosses, like nested association mapping (NAM). QTL allele effects for MPPs can be modelled in various ways. For example, these effects can be related to a specific parent. Another way of defining QTL effects is to link them to marker score. In between these extreme, alleles can also come from ancestral lines and be shared by several parents. Polygenic effects in MPPs can have homogeneous or heterogeneous variance. We compare model to perform QTL detection in MPPs taking into consideration the different sorts of QTL effects and heterogeneity of polygenic variance. We illustrate our approach using various subsets of the maize EU-NAM data.

Several papers already proposed models with different types of QTL effects. These models generally include a single type of QTL effect (e.g. cross-specific or bi-allelic). It is however more reasonable to assume that QTL effects are diverse. Therefore, we propose a solution to combine different types of effect and build models allowing each QTL position to have a different form. According to our results this strategy detect positions taking better into consideration the variability present in the population.

In addition, we propose to replace the classical assumption of homogeneous residual term by cross-specific residual terms. From a theoretical point of view, the use of cross-specific terms is more appropriate because it respects the variability structure present in the different parts of the population. When heterogeneity is high, the homogeneous error term assumption will cause false positive and penalize effects that are in fact more certain. We show in our results that in situation characterized by a high level of polygenic effect (many small undetected effects), the use of cross-specific error terms can help to detect extra QTL position and account for a higher proportion of phenotypic variance.

In conclusion, the combination of different types of QTL effects and the use of cross-specific residual terms are two elements allowing to increase the accuracy of the QTL detection in MPPs. The presented models have been implemented in an R package called mppR.

Molecular characterization of the cytoplasmic male sterility system underlying the breeding and production of Hyvido^{tm} hybrids in barley

Carine Rizzolatti[a], Nathalie Rodde[b], Hélène Bergès[b], Edouard Tatara[c], Pierre A. Pin[a], Françoise Budar[d], Hakim Mireau[d], Paul Bury[e] and Jan J.L. Gielen[a]

[a] *Genetics Department, Syngenta, France*
[b] *Centre National de Ressources Génomiques Végétales (CNRGV), INRA, France*
[c] *Cereals Breeding Department, Syngenta, France*
[d] *Institut Jean-Pierre Bourgin (IJPB), INRA, France*
[e] *Cereals Breeding Department, Syngenta, United Kingdom*

carine.rizzolatti@syngenta.com

Hybrid technology has proven a valuable production system in many crops, but was only recently adopted in cereals. Syngenta is commercializing hybrid varieties in barley (*Hordeum vulgare*) using the Cytoplasmic Male Sterility (CMS) system derived from *Hordeum vulgare ssp spontaneum* (Ahokas, 1979). To gain further insight into mechanisms underlying both the CMS and the male fertility restoration, we used two complementary approaches to identify the molecular components involved in this hybridization system.

For the purpose of identifying the sterility-inducing factor, we sequenced the entire mitochondrial genome in both barley and *spontaneum*. Plant mitochondrial genomes are repeat-rich and highly dynamic in structure, which renders their sequence assembly rather challenging. Using Illumina paired-end and mate-pair sequence reads, complemented with PacBio sequences, the *de novo* assembly of the entire genomes for both the sterile and fertile cytoplasms was achieved. The annotation and comparative analysis of these sequences allowed us to select a list of open reading frames specific for the sterility-inducing cytoplasm. A strong candidate for the sterility factor was subsequently identified by virtue of its expression profile and its chimeric nature.

Taking advantage of the available genomic resources in barley, in combination with a custom-made non-gridded BAC library developed from a restorer line, we also managed to clone the restorer gene *Rfm1* mapping to the top of chromosome 6H. *Rfm1* belongs to the PLS-DYW subfamily of PPR proteins that are known for their involvement in RNA editing in plants organelles, but that to date have not been identified as restorer genes. We will describe the structural complexity of the *Rfm1* locus and the potential mode of action of *Rfm1*.

Ahokas H., (1979), Cytoplasmic male sterility in barley. Acta Agric Scand 29:219–224

Utilizing low-coverage sequence data in tomato recombinant inbred lines (*S. lycopersicum x S. pimpinellifolium*)

Rianne van Binsbergen[ab], Marco C.A.M. Bink[c], Richard Finkers[d], Mario P.L. Calus[b], Roel F. Veerkamp[b] and Fred A. van Eeuwijk[a]

[a] *Biometris, Wageningen UR, The Netherlands*
[b] *Animal Breeding and Genomics Centre, Wageningen UR, The Netherlands*
[c] *Hendrix Genetics Research, Technology & Services B.V., The Netherlands*
[d] *Wageningen UR Plant Breeding, Wageningen UR, The Netherlands*

rianne.vanbinsbergen@wur.nl

The availability of the *Solanum Lycopersicum* 'Heinz 1706' reference genome (The Tomato Genome Consortium (2012)), together with rapid development of next generation sequencing techniques provides opportunities for modern tomato breeding. A cost effective approach would be to use 'old' SNP array genotyping data, together with genotype imputation to obtain sequence data information for large segregating populations. Building on these developments, the first objective of our study was to perform genotype imputation and asses the accuracy in a tomato RIL population. A second objective was to investigate the added value of (imputed) whole-genome sequence data relative to a SNP array when performing QTL linkage analysis.
A set of 51 RILs (F8) was available from a cross between *S. lycopersicum* (cv. Money maker) and *S. pimpinellifolium* G1.1554, which were sequenced at low-coverage (Viquez-Zamora et al. (2014)), with a mean sequencing depth of 7.3. For both parents sequence data were also available. All sequence genotypes of the RIL population were masked, except for the positions that were present on a custom made SNP array (Víquez-Zamora et al. (2013)). These masked SNPs were imputed using PlantImpute (Hickey et al. (2015)). Imputation accuracy was assessed as the proportion of SNP genotypes imputed correctly. QTL linkage analyses were performed per SNP using an independent two-sample t-test, assuming equal variances. The studied traits were average fruit weight and soluble solid content (brix).
Based on regression coefficients between SNP positions on the physical and genetic maps, each chromosome was divided into three regions: left- and right arm, and centromere. The sequence data consisted of 2,787,027 SNPs with 83.4% located in the centromere region. This high percentage is in contrast to the SNP array, which consisted of 1663 SNPs with 34.9% in the centromere region. Due to computational issues, we were only able to run PlantImpute for the chromosome arms. The number of SNPs per arm ranged between 7,002 and 46,630 SNPs on sequence data, and between 5 and 166 on SNP array. The largest arms were split in two runs of maximal 25,000 sequenced SNPs. Per run between 24.5 % and 97.4% of the genotypes (across all SNPs and individuals) were imputed, with an average proportion imputed correctly between 0.879 and 0.999.
Linkage analyses showed several significant QTL regions ($P < 0.01$) that were consistent across the three data sources (SNP array data, imputed sequence data, and true sequence data). In addition, QTL regions were detected when using (imputed) sequence data, which were not found with SNP array data. Although the number of observations is very low (~50), the imputation and QTL results suggest that (imputed) low coverage sequence data could have additional value to modern plant breeding.

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Plenary session 2a:
Abiotic stress tolerance

Making genotype x environment interaction accessible to breeding for drought resistance

Pauline Thomelin[a], Fabio Arsego[a], Habtamu S Tora[a], Penny Tricker[a], Fahimeh Shahinnia [b], Paul Eckermann[a] and **Delphine Fleury[a]**

[a] *Australian Centre for Plant Functional Genomics, University of Adelaide, Australia*
[b] *Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany*

delphine.fleury@acpfg.com.au

Drought and heat are major concerns for crop production as they dramatically reduce grain yield. Due to the increasing occurrence of drought and heat particularly in the Mediterranean region, the USA, India and Australia, yield improvement under these stresses is a priority. Yield is a complex quantitative trait whose expression is highly influenced by the environment and agronomic management. Breeding for drought tolerance is further complicated since several types of abiotic stress, such as high temperatures, high irradiance and nutrient toxicities or deficiencies can challenge crop plants simultaneously. A research program for increasing drought tolerance of in cereals should tackle the problem in a multi-disciplinary approach, considering interaction between multiple stresses and plant phenology, and integrating the physiological dissection of drought tolerance traits and the genetic and genomics tools.
A way to improve drought tolerance in crops is to discover new genes and alleles that allow plants to continue to grow and yield grain under water limited conditions. Although Quantitative Trait Loci (QTL) have been identified in cereals for yield in dry environments, most of those are "unstable" across environments which makes them difficult to use breeding without detailed information on their function. Over ten years program, the ACPFG has cumulated QTL information on wheat populations for yield, agronomical, morphological and physiological traits in various locations in Australia, India and Mexico, and under controlled conditions. By coupling physiological and genetic methods we dissected the responses to the environment and identified the conditions where some specific QTL are expressed. Such detailed information is crucial for breeding application.

Obstacles and challenges when breeding for nitrogen use efficiency in vegetable crops

Edith T. Lammerts van Bueren[a] and Paul C. Struik[b]
[a] *Plant Breeding Wageningen UR, Wageningen University, Wageningen, The Netherlands*
[b] *Centre for Systems Analysis, Wageningen University, Wageningen, The Netherlands*

edith.lammertsvanbueren@wur.nl

Sustainable cropping systems require careful nutrient management based on relatively low supply and optimal use efficiency. This is especially true for nitrogen as it is very mobile, pollutes the environment and the production nitrogen fertiliser requires a lot of fossil energy. Nitrogen is in high demand by the crop and designing crop systems with high productivity but low nitrogen input is a challenge. Agronomists aim at improving nitrogen management of an individual crop but also at designing an optimal rotation in which nitrogen is kept in the system and passed on to the various succeeding crops. Such strategies are challenging as they require coping with variable, and increasingly unpredictable environmental conditions. Breeders can contribute to meeting this challenge by improving the nitrogen use efficiency (NUE) within a crop species, i.e. by increasing the total biomass produced per unit N supplied. NUE is a complex trait comprising two key components: the uptake efficiency (NUpE) and the utilisation efficiency (NUtE) per unit N taken up.

Breeders are interested in developing cultivars that are nutrient efficient under low-input conditions but responsive when more nutrients are available. When breeding for NUE, the following questions are relevant: i) which (combinations of) traits contribute to NUE?, ii) can these traits be phenotyped efficiently?, iii) which of these traits show sufficient variation and heritability for efficient selection?, iv) under what growing conditions is the genetic variation in these traits best expressed and can one best select?

Most research on improving NUE in crops has focused on cereals and oil seed crops. We will review NUE in leafy (spinach, lettuce, cabbage) and non-leafy (potato) vegetable crops. Short cycle crops that need to produce biomass in a short time differ in their nitrogen capture strategy from long cycle crops that have more time to overcome fluctuating nitrogen availability. The various vegetables also differ in nitrogen reallocation strategies and the extent in which reallocation plays a role in producing high-quality and healthy produce.

Research into the genetic variation of NUE related traits is complicated by the fact that the results differ for different growing types or maturity types. In potato we found that NUE is strongly correlated to maturity type: late-maturing cultivars are more efficient than early-maturing or intermediate cultivars. Fortunately, there is considerable genetic variation to select for NUE genotypes within each maturity type. The same was found for cabbage. In spinach, fast- and slow-growing cultivars differ in their strategy to cope with limiting N availability.

Most research on vegetables crops, such as lettuce and spinach, showed that under field conditions the impact of environmental variation was larger than that of the genotypic variation. Recommendations to identify effective selection traits while coping with the large genotype × environment interaction point into different directions: a) developing a controlled system for (early) selection such as a hydroponic system, or b) the need to develop an ecophysiological model to assist breeders in their selection process.

However, the final selection always needs to be done under field conditions, preferably under conditions of both low and high nitrogen input, as cultivars can differ in their mechanisms to cope with nitrogen shortage and abundance in various growing conditions.

Genome wide association mapping approach searching for frost tolerance in wheat (*triticum aestivum* l.)

Ulrike Lohwasser[a], Fernando Arana-Ceballos[a], Michael Koch[b], Kerstin Neumann[a] and Andreas Börner[a]

[a] *Leibniz Institute of Plant Genetics and Crop Plant Research Gatersleben (IPK), Genebank Department, Germany*
[b] *Deutsche Saatveredelung AG (DSV), Germany*

lohwasser@ipk-gatersleben.de

Wheat is one of the most important crops for human nutrition and is the most widely grown cereal crop in the world. Wheat yield stability has to be ensured in the context of climate change. Frost tolerance is essential for autumn-planted wheat to survive freezing temperatures during winter in temperate zones. It is a key trait with economic and agronomic importance but its molecular and genetic background (a complex trait with polygenic inheritance) is still poorly understood. The aims of the present study are (1) to investigate phenotypic variation for frost tolerance in a panel of 276 winter wheat accessions (2) to generate information about genetic variation and (3) to find molecular markers closely linked to the trait frost tolerance. Frost tolerance phenotype scores were collected from several locations in Germany and Russia during two seasons and were combined with the genotypic data in genome wide association studies (GWAS). The genotyping was done employing ILLUMINA infinium iSelect 90k wheat chip. The chip carries a total of 81,587 valid and functional SNPs. Finally, 16,000 polymorphic markers could be used for the genome wide analyses. SNP associations were performed using linear mixed models that evaluated the effects of SNPs with minor allele frequencies (MAF) > 10% individually, adjusting for population structure and kinship. For the population structure analysis, the Q-matrix for three groups was chosen as the best option; subsequent validation confirmed its results and using an evolutionary tree calculated by the software 'PAUP', three subgroups of North American, Russian and North and Middle European genotypes were detected. GWAS analyses of the most significant SNP loci (highly significant associations (LOD>3) identified three and seven positive SNP associations on the chromosomes 1B and 5A, respectively. Haplotype analysis revealed that most of the significant SNP loci for these positions represent an advantage for the evaluated genotypes. Validation of SNPs and their respective haplotypes associated with frost tolerance together with candidate gene based-association studies, will be performed in future studies to determine the diagnostic value of markers for marker assisted selection in winter wheat breeding programs.

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Tomato likes it hot, but pollen not - understanding heat-tolerance of male fertility in tomato

Nicky Driedonks[a] , Jiemeng Xu[a] , Florian Müller[a], Celestina Mariani[a] and **Ivo Rieu[a]**

[a] *Dept Molecular Plant Physiology, Radboud University, The Netherlands*

i.rieu@science.ru.nl

High ambient temperature imposes constraints on plant reproductive success and thus markedly affects crop yield. Using modelling of tomato fruit and seed set under prolonged moderately elevated temperature we showed that reduction of male fertility is a major limiting factor. Pollen development was found to be heat sensitive around meiosis and early microsporogenesis, although defects in subsequent pollen development became apparent at a much later stage only. Gene expression and mutant phenotype analysis showed that loss of male organ identity contributes to heat-induced pollen defects. Phenotyping of a set of cultivated and wild tomato species revealed substantial natural variation for many putative sub-traits of reproductive success. A number of (partially identical) additive and interacting heat tolerance QTLs were identified in various bi-parental F2 populations, which alone or in combination may prove useful for heat tolerance breeding. Identification of the underlying genes and their function, by fine-mapping and transcriptome profiling, will further add urgently necessary insight into the processes limiting pollen development under high temperature.

Pup1 and beyond: Developing rice adapted to infertile soils in Africa

Matthias Wissuwa[a], Katsuhiko Kondo[a], Juan Pariasca-Tanaka[a], Joong H Chin[b] and Khady N Dramé[c]

[a] *Japan International Research Center for Agricultural Sciences (JIRCAS), Japan*
[b] *Institute/Department, Research Station, Korea*
[c] *Africa Rice Center (AfricaRice), East and Southern Africa regional station, Tanzania*

wissuwa@affrc.go.jp

Improving nutrient uptake has long been an objective in crop breeding programs, especially in tropical areas where less fertile soils dominate and farmers often have limited resources to improve soil fertility through fertilizer application. We have previously identified *Pup1*, a major QTL for phosphorus (P) uptake, and after cloning of the underlying gene *OsPSTOL1*, showed that it enhances P uptake through better root development under P deficient conditions (Gamuyao et al. 2012). The *Pup1* locus has been introgressed into widely grown upland and lowland rice varieties through marker assisted selection and we will report on the progress of *Pup1* breeding activities in Africa.

Maintaining relatively high root growth rates during nutrient deficiencies is only one of the possible adaptation strategies. Additional tolerance mechanisms to enhance plant performance on low-P soils could be exploited if genotypic variation existed. We recently targeted two such mechanisms: Root Efficiency (RE) which we define as the amount of P taken up per unit root size; and internal P utilization efficiency (PUE) which essentially refers to the ability to produce biomass and maintain normal plant function at reduced tissue P concentrations. Genome wide association studies (GWAS) using an association panel consisting of a global collection of rice accessions detected loci for PUE on chromosome 1 within the *indica* subspecies, and on chromosome 11 for the *aus* subspecies (Wissuwa et al. 2015). In both cases a rare haplotype enhanced PUE and donors have been identified. RE was evaluated in field experiments following a 'shovelomics' protocol and one promising locus was identified on chromosome 11 (Mori et al. 2016). Again, one *aus*-specific rare haplotype was identified with a suitable donor. For both novel traits, donors were crossed to popular rice varieties lacking the rare alleles and our medium-term goal is the pyramiding of all three complementary P efficiency traits.

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Flash and poster presentations: Cross-cutting topics

Better breeding decision using high throughput de novo assembly and advanced genomic big data analytics

Kobi Baruch [a]

[a] *NRGene, Israel*

kobi@nrgene.com

Next Generation sequencing technologies have opened the door to multiple genome analyses and an increased understanding of the variation present in the genomes of plants. To date, most of the germplasm analyses have relied on the comparison of sequence reads to a reference assembly of a representative accession limiting our understanding of genome variation to SNPs and small Indels. This talk will present the next generation of genomic analysis that is based on the full genomic sequence of the entire germplasm. This task is accomplished by two complementary products by NRGene: DeNovoMAGIC, a denovo sequence assembler of complex genomes, which enable cost effective production of reference quality genomes from many diverse lines of a single species such as wheat and maize, and GenoMAGIC, big data analytics software, which enable all-by-all genomes comparison and high resolution haplotyping of the entire germplasm. This talk will introduce the application of this technology to build a comprehensive genomic database for maize that reveal the full breadth of variation complexity and is utilized to accelerate breeding, marker assisted selection and trait mapping.

Localization of metabolites in plant tissues using high-resolution mass spectrometry imaging

Dhaka Ram Bhandari[a], Bin Li[b], Sven Gottwald[c], Andreas Römpp[d] and Bernhard Spengler[a]

[a] *Institute of Inorganic and Analytical Chemistry, Justus Liebig University Giessen, Germany*
[b] *Department of Chemistry, University of Illinois, USA*
[c] *Department of Plant Breeding, Justus Liebig University Giessen, Germany*
[d] *Chair of Bioanalytical Sciences and Food Analysis, University of Bayreuth, Germany*

Dhaka.R.Bhandari@anorg.Chemie.uni-giessen.de

Mass spectrometry imaging (MSI) is a label-free chemical imaging technique. This method provides spatial information of a large variety of analytes from a single tissue section. Recently, MS imaging has been used to perform non-targeted metabolomics, lipidomics and proteomics studies. This tool has been utilized to investigate spatial distributions of endogenous, metabolic and drug molecules in insect whole body sections, plants, human and mammalian tissues [1]. To resolve the complexity of biological samples, at high mass resolution, an improved mass accuracy and a subcellular spatial resolution are essential. Here we use an atmospheric-pressure scanning microprobe matrix-assisted laser desorption ionization mass spectrometry imaging (AP-SMALDI MSI) system with high resolution in mass and space, for its novel application in plant tissue obtained from crop plants *Brassica napus* (rapeseed) and *Triticum aestivum* (wheat seed) and the medicinal plant *Paeonia lactiflora* (peony). Sample preparation is one of the major challenges to obtain high-quality MS images. Several tissue-sectioning methods (cryosectioning, embedding, tape-assisted methods etc.), compatible with MS imaging experiments, were optimized. A high-resolution matrix-preparation unit was used to apply the matrix (SMALDIPrep, TransMIT GmbH, Giessen, Germany). A high-resolution atmospheric pressure scanning microprobe matrix-assisted laser desorption/ionization imaging source (AP-SMALDI10, TransMIT GmbH, Giessen, Germany) coupled to a Fourier transform orbital trapping mass spectrometer (Q Exactive, Thermo Scientific GmbH, Bremen, Germany) was used to rasterize the tissue section [1]. High-quality *m/z* images (bin width of ± 5 ppm), showing highly resolved features down to 5 µm pixel size, were generated using the software package 'Mirion'. MS images were generated using the AP-SMALDI10 system to understand the metabolic changes during germination of oilseed rape and after fungal infection in wheat seed. In a single experiment, more than 90 compounds were visualized. Metabolites of the sinapate ester metabolism, having similar chemical structures, were detected and visualized. Visualizing such metabolites simultaneously would be practically impossible with classical visualization techniques. In case of fungus-infected wheat seeds, metabolites such as trimethylammonio butanoate, related to fungal infection, were detected and localized. In peony, major tissue-specific metabolites, including monoterpene glucosides and gallotannins, were successfully visualized at the cellular level. AP-SMALDI MSI provides efficient technological advancements in the visualization of individual molecular species in plant tissue sections.

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A new lab guide on genotyping-by-sequencing for plant genetic diversity analysis

Yong-Bi Fu

Plant Gene Resources of Canada, Saskatoon Research and Development Centre
Agriculture and Agri-Food, Canada

yong-bi.fu@agr.gc.ca

There are more than 7 million plant germplasm samples conserved in 1,700 seed banks worldwide. These are valuable as virtually untapped and unlimited genetic sources of agronomic and quality traits. To accelerate the search for desirable plants from these collections, an effective characterization tool is needed to mine genetic variants across plant genome.

Genotyping-by-sequencing (GBS) has emerged as a promising genomic approach for exploring and characterizing plant genetic diversity on a genome-wide scale. However, many uncertainties and challenges remain in the GBS applications, particularly in non-model species. We develop a genetic diversity focused GBS (gd-GBS) protocol and present it as an easy-to-follow lab guide to assist a researcher through every step of a GBS application from sample preparation, library assembly, sequencing, SNP calling, to diversity analysis. It uses two restriction enzymes to reduce genome complexity, applies Illumina multiplexing indexes for barcoding, and has a custom bioinformatics pipeline (npGeno) for automatic SNP genotyping. In this presentation, I will introduce the new GBS lab guide, illustrate its application, discuss related application issues, and update our current efforts in GBS research. Following these lab bench procedures and using the npGeno pipeline, one could generate genome-wide SNP genotype data for a conventional genetic diversity analysis of a plant species.

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Applying segregation distortion approach in QTL analysis of three non-BSSS doubled haploid populations in maize

Maja Mazur[a], Sonja Marić[b], Ivan Brkić[a] and Domagoj Šimić[a]

[a] *Agricultural Institute Osijek, Croatia*
[b] *Faculty of Agriculture, University of Osijek, Croatia*

maja.mazur@poljinos.hr

Three biparental populations of doubled haploid (DH) maize lines (Os2702, Os2703 and Os2709) derived by *in vivo* haploid induction technique from crosses of five maize dent non-BSSS (stiff stalk) inbred lines were studied for flowering time. We applied novel method for QTL mapping via segregation distortion based on generalized linear mixed model in multiple breeding populations requiring no large population sample. Population sizes ranged from 33 individuals (Os2702) to 68 individuals (Os2703). The populations were genotyped by using the Illumina MaizeSNP50 BeadChip. Across all three populations, distorted markers were observed on all chromosomes, but certain chromosome regions were more affected than others. We focused on a segregation distortion region (SDR) on chromosome 3 (bin 3.9) detected by significant joint Wald test statistics in all three DH populations. SNP markers with the most extreme segregation distortion in the SDR in respective populations were associated with anthesis calculated as growing degree days (GDD). In average, GDD from emergence to anthesis were 785, 800 and 808 in three populations respectively. However, the SDR was mapped nearby to the known gametophyte factor *ga7* suggesting that the factor may be a genetic reason for segregation distortion. Our further investigations on other SDRs of the maize genome might elucidate the effectiveness of QTL mapping by detecting segregation distortion.

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BRIDGE: Biodiversity informatics for harnessing barley genetic diversity hosted at the genebank of IPK Gatersleben

Sara G. Milner, Axel Himmelbach, Markus Oppermann, Stephan Weise, Andreas Graner, Helmut Knüpffer, Uwe Scholz, Andreas Börner, Martin Mascher and Nils Stein

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Germany

milner@ipk-gatersleben.de

Genomics and biodiversity informatics are rising as fundamental tools to harness genetic resources harbored in germplasm collections, which represent essential albeit mostly untapped reservoirs of genetic diversity for crop research and improvement. The BRIDGE project aims to molecularly characterize more than 20,000 accessions of domesticated (*Hordeum vulgare L.* subsp. *vulgare*) and wild (*Hordeum vulgare* L. subsp. *spontaneum* (K. Koch) Thell.) barley hosted at the German *ex situ* genebank at IPK Gatersleben, by means of a genotyping-by-sequencing (GBS) approach. GBS data will be analyzed in context of the barley genomic framework to study population structure and genetic diversity patterns and also to mine allelic variation at breeding-relevant traits. A novel warehouse infrastructure will provide a systematic valorization to the upcoming genomics data and a link to the passport and phenotypic data accumulated by the IPK genebank conservation management. Here, we describe our bioinformatics pipeline for read mapping, variant detection and genotyping. First results of the GBS analysis conducted on more than 6,000 barley accessions will be presented.

Development and characterization of a new wheat-rye Robertsonian translocation with *Sr59* resistance to stem rust

Mahbubjon Rahmatov[a], Matthew N. Rouse[b], Tatiana Danilova[c], Bernd Friebe[c], Brian J. Steffenson[d], and Eva Johansson[a]

[a]*Swedish University of Agricultural Sciences, Department of Plant Breeding, Alnarp, Sweden*
[b]*United States Department of Agriculture, Agricultural Research Service, Cereal Disease Laboratory, Minnesota, USA*
[c]*Kansas State University, Department of Plant Pathology, Kansas, USA;*
[d]*University of Minnesota, Department of Plant Pathology, Minnesota, USA*

mahbubjon@gmail.com

Stem rust of wheat caused by the fungus *Puccinia graminis* f. sp. *tritici* Eriks. and Henn. is one of the most important threats to wheat production worldwide. The new widely virulent group of stem rust races in the Ug99 lineage (race TTKSK) originating from East Africa has caused great concern for wheat food security. In addition, emergence and spread of race TKTTF to Germany, Sweden and Denmark is a threat to the European wheat production. In this context characterization and deployment of new resistance sources against stem rust is a high priority. The objectives of this study were to identify new sources of resistance in wheat-*Secale cereale* derivatives. A large number of wheat-rye introgression lines were developed during 1980-2000 at the Swedish University of Agricultural Sciences in Sweden. These wheat-rye introgression lines were screened with a wide array of stem rust races at the seedling and adult stages. In addition molecular and cytogenetics analysis were applied. The results identified three 2R (2D) wheat-rye substitution lines (SLU210, SLU238 and SLU239) as new sources of stem rust resistance gene/s. Thereafter, the SLU238 line was crossed with the Chinese Spring *ph1b* mutant that induces meiotic pairing between homoeologous chromosomes between 2D and 2R wheat and rye chromosomes. The progeny was evaluated for presence of reduced rye chromatin through stem rust seedling resistance screening, molecular and cytogenetic analysis. A homozygous T2DS·2RL wheat-rye translocation was obtained through the breakage-fusion mechanism, and designated as the source of *Sr59* in the wheat genome. The characterization of *Sr59* and its introgressing through 2RL into wheat provides wheat breeders with an additional genetic resource for breeding for stem rust resistance. *Sr59* can be pyramided with other stem rust resistance genes using the Kompetitive Allele Specific PCR (KASP) markers identified in this study to select for wheat lines with multiple stem rust resistance genes.

How next-generation sequencing can improve and speed up breeding of new crop varieties

Leonie D. Fritsch[a], Stefan Schillberg[a,b] and **Florian Schröper[a]**

[a] *Fraunhofer-Institute for Molecular Biology and Applied Ecology IME, Aachen, Germany*
[b] *Phytopathology Department, Institute for Phytopathology and Applied Zoology, Justus-Liebig University Giessen, Germany*

florian.schroeper@ime.fraunhofer.de

The continuously growing world population and dwindling resources necessitate the generation of new varieties of staple crops to increase productivity without the need for additional land or chemical inputs. Hence, breeders seek more efficient approaches to generate better adapted crop varieties that remain productive under adverse conditions. The speed and efficiency of plant breeding can be improved by adopting SMART breeding technologies and genetic modifications, all of which have advantages and disadvantages and can underlie strict regulations in various countries. Modern breeding techniques are steadily replacing or augmenting classical breeding approaches. Unfortunately current SMART breeding approaches cannot determine zygosity. Only a reliable identification of relevant genomic regions that correspond to a trait of interest and their allelic distribution, can achieve an effective selection of young plants for further propagation.

Next-generation sequencing (NGS) can be used to generate millions of short reads spanning various loci and can therefore be used to map numerous different quantitative trait loci (QTL) and their specific allelic diversity. NGS has been applied in human genetics to identify disease alleles and polymorphisms in populations. Similar NGS based concepts can be used to even gain new insight into the genomic structure of plants, facilitating completely new SMART breeding approaches.

Here we present a NGS based technique which supports conventional breeding of natural traits with high throughput and efficiency. The technique enables to give a consistent and unambiguous statement concerning selected hereditary phenotypic traits, which are fine mapped to certain genes and polymorphisms, and their zygosity. This information can then be used for efficient plant selection during a highly targeted breeding processes, which lead to a faster generation of new and improved plant lines with predictable success. Barley and maize were selected as model plants due to their economic importance, the availability of adequate amounts of reference sequence data, and their diploid genome. One major aspect of the project was the applicability of the assay to a large number of plants in parallel while keeping the cost at a modest price.

Barley and maize hybrids and their corresponding parental lines were analysed with respect to a number of different preselected traits such as resistance to certain pathogens, tolerance to abiotic factors as well as their nutritional value. In all cases known QTL in the genome were amplified in a special multiplex PCR format for up to 96 seedlings. All amplicons where pooled and sequenced using NGS. Barcodes were employed to distinguish individual plants. NGS data was then analysed to identify individual seedlings carrying genetic marker sequences such as SNPs or InDels associated with the desired phenotype. Utilizing this novel technique enables to reliably determine the genetic composition with respect to QTL and their allelic distribution for a large number of hybrids. It thus facilitates to predict their phenotype at a very early stage allowing to select promising candidates for further propagation and subsequent breeding steps.

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Improvement of plant growth and stress tolerance using cyanobacterial flavodi-iron proteins (FDPs)

Fahimeh Shahinnia[a], Suresh Tula[a], Goetz Hensel[a], Nicolaus von Wirén[a], Nestor Carrillo[b], Rodrigo Gomez[b] and Mohammad-Reza Hajirezaei[a]

[a] *Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK), Department of Plant Physiology and Cell Biology, Germany*
[b] *Instituto de Biologıa Molecular y Celular de Rosario (IBR, CONICET), Universidad Nacional de Rosario, Argentina*

shahinnia@ipk-gatersleben.de

Environmental stresses and nutrient limitation are among the major causes for crop losses worldwide. Engineering strategies aimed at improving growth and stress tolerance have mostly focused on overexpression of plant-endogenous genes belonging to molecular networks for stress perception or stress responses. A new alternative approach has recently been applied with considerable success to model plants. It is based on the replacement of stress-sensitive plant targets such as ferredoxin by stress-resistant cyanobacterial flavodoxin. The expression of cyanobacterial flavodoxin in chloroplasts in transgenic tobacco plants led to tolerance to various stresses including drought and iron starvation, thus representing a biotechnological application for the generation of crops tolerant to multiple stresses. In the present study, we have tested additional cyanobacterial proteins, flavodi-iron proteins (FDPs), for further investigation of this approach. FDPs are widely distributed among bacteria, archaea and cyanobacteria and show various properties such as dioxygen-scavenging reductase and nitric oxide-scavenging reductase activity indicating a response to oxidative stress superior to that of flavodoxin. In Cyanobacteria, FDPs are encoded by four different genes Flv1, Flv2, Flv3 and Flv4 and function in tandem. Further, FDPs are a sub-class of redox-active proteins containing Fe in a form that does not occur in higher plant proteins. We have introduced the Flv genes in the genome of the plant species *Arabidopsis*, tobacco and barley to identify the underlying molecular and biochemical mechanisms for their protective action against oxidative stress and to identify the environmental and nutrient conditions, under which Flv-mediated stress tolerance allows for biomass and yield enhancement.

RABBIT: reconstructing ancestry blocks bit by bit in experimental populations

Chaozhi Zheng[a], Martin P. Boer[a] and Fred A. van Eeuwijk[a]

[a] *Biometris, Wageningen University and Research Centre, The Netherlands*

chaozhi.zheng@wur.nl

We present a general hidden Markov model framework called RABBIT for reconstructing genome ancestry blocks from single nucleotide polymorphism (SNP) array data, a necessary step for quantitative trait locus (QTL) mapping. RABBIT is highly flexible. (1) It can be applied to a stage-wise random mating population, a population with a fixed breeding pedigree, and a population without any information on breeding design (apart from the genotypic data of inbred founders and offspring). Examples include the *Arabidopsis* multiparent advanced generation intercross (MAGIC), and the funnel-type crop MAGIC populations (e.g. rice, wheat, tomato). (2) It can be applied to a mapping population with complete, partial, or no inbreeding. (3) It can account for missing data and allelic typing errors in the genotypic data of founders and sampled offspring. Studies on simulated and real data of the *Arabidopsis* MAGIC demonstrate that RABBIT is more robust and accurate in reconstructing genome ancestry blocks than some commonly used methods. RABBIT is publicly available on Github at https://github.com/chaozhi/RABBIT.git.

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Genetic gains in bi-parental population improved through marker assisted recurrent selection under drought stress

Rekiya O. Abdulmalik[a,b], Abebe Menkir[a], Melaku Gedil[a], Shehu G. Ado[b], Joseph D. Olarewaju[b], Daniel A. Aba[b], Muhyideen Oyekunle[b], Sarah Hearne[c] and Jose Crossa[c]

[a] *International Institute of Tropical Agriculture (IITA), Nigeria*
[b] *Institute for Agricultural Research (IAR), Ahmadu Bello University, Nigeria*
[c] *International Maize and Wheat Improvement Center (CIMMYT), Mexico*

rekimalik83@yahoo.com

Marker assisted recurrent selection (MARS) has been proposed as a breeding strategy to increase the frequency of favorable alleles that confer tolerance to drought in inbred lines derived from bi-parental crosses. Assessment of genetic gain in a bi-parental cross subjected to MARS is therefore important to determine the usefulness of this approach for inbred line improvement. A bi-parental population involving a pair of drought tolerant and *Striga* resistant maize inbred lines improved through two cycles of MARS was the source of 200 S_1 lines that were crossed to a tester belonging to a complementary heterotic group. The resulting testcrosses were evaluated under carefully controlled drought stress at Ikenne and under artificial *Striga* infested and *Striga*-free conditions at Abuja and Mokwa in Nigeria for two years. The 200 S_1 lines were also genotyped using 233 single nucleotide polymorphism markers. The average gain from MARS for grain yield was 53 kg ha^{-1} under drought stress and 117 kg ha^{-1} under *Striga* infestation. The changes in frequency of favorable alleles increased with advances in selection from 0.50 at C_0 to 0.52 at C_2 in the bi-parental population with none of the markers showing fixation. The study demonstrated the effectiveness of MARS in enhancing genetic gain and increasing the frequency of favorable alleles for tolerance to drought in a bi-parental population targeted as a source of improvement of maize inbred lines.

Organic vegetable breeding at Sativa Rheinau

Charlotte Aichholz[a], Friedemann Ebner[a] and Noémi Uehlinger[a]

[a] *Sativa Rheinau AG, Switzerland*

c.aichholz@sativa-rheinau.ch

Organic growers need varieties that are bred for the specific needs of organic farming systems. Furthermore, organic growers aim at supporting the whole agro-ecological system through stimulation of self-regulating processes. In this context, there is a need for plant varieties which combine general robustness, horizontal resistance, high resilience and good yield stability under organic conditions. Sativa Rheinau breeds for open-pollinated varieties adapted to organic conditions. All selection steps are done under organic field conditions in order to assess the overall robustness of plants or breeding lines. The main breeding goals are a high marketable yield which is constant over the years, a good robustness towards biotic and abiotic stresses, as well as good taste. After 12 years of work, the first varieties of extra-sweet maize, carrots, kohlrabi, eggplant and onions are now commercially available.

Innovative approaches to optimize genetic diversity for sustainable farming systems of the future (INSUSFAR) – Project presentation

J. Peter Baresel[a], Gunther Backes[c], Lorenz Bülow[f], Maria R. Finckh[e], Lothar Frese[f], **Samuel Knapp[a]**, Detlev Möller[d], Hans-Jürgen Reents[b], Torsten Siegmeier[d], Robert Simon[b], and Odette Weedon[e]

[a] Chair of Plant Nutrition, [b] Chair of Organic Farming and Agronomy, Technische Universität München, Germany
[c] Department of Organic Plant Breeding and Agrobiodiversity, [d] Department of Farm Management, [e] Department of Ecological Plant Protection, University of Kassel, Germany
[f] Institute for Breeding Research on Agricultural Crops, Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Germany

samuel.knapp@tum.de

Sustainable agricultural systems need to reduce the dependence on external inputs while maintaining or increasing overall system output including delivery of ecological services. Among other means, functional diversity at the within- and between-species levels can be a major component. However, there is a lack of knowledge about the optimum level of diversity needed for high yield and yield stability, while at the same time achieving maximum self-regulation to reduce the necessary inputs.
Thus, the overall aim of the INSUSFAR project is to generate a better understanding of how genetic diversity in self-pollinating crops using wheat as a model species can be optimized with respect to inputs, ecological services, and economic outputs. In particular, the following questions will be addressed:
(1) Has breeding so far contributed to the adaptation of varieties to more sustainable systems? (2) What are the effects of varying degrees of within-crop diversity and different morphological types on the adaptability to different agricultural systems? (3) How does diversity develop over time in different types of systems and which selection and maintenance methods are thus needed to improve the benefits of diversity? (4) What is the potential socio-economic and ecological impact of more diverse crops in sustainable agricultural systems?
To answer these questions, (1) data from variety testing in Germany will be analysed for the effect of breeding progress on the adaptation to agricultural systems differing in input, and a meta-analysis of published studies on breeding progress will be carried out. (2) The performance of differently adapted populations and genotypes of different morphological type will be investigated in cropping systems with different levels of input and diversity (living mulch). Experiments will be carried out as standard field trials and in established cropping systems (medium-term-experiments and selected farms). (3) The genetic changes of genetically diverse populations will be studied. (4) Material and energetic fluxes, as well as indicators for sustainability will be analysed in detail and results will be evaluated for the socio-economic and ecological impact. In addition, scenarios will be calculated/simulated on rotation, farm and regional levels.
The results will be reflected for their potential effects on agricultural practices as well as the political and administrative measures that might be necessary to support sustainable agricultural development. As breeding is a long-term process, it will be ensured that the data generated in the project will be available for future research as public source in a newly developed database.

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Single cell genomic sequencing in *Brassica napus* and wheat: Applications in monitoring recombination frequency

Venkatesh Bollina[a], Pankaj Bhowmik[a], Erin Higgins[b], Patricia L. Polowick[a], Alison M.R. Ferrie[a], Isobel A.P. Parkin[b], Andrew G. Sharpe[a] and **Sateesh Kagale[a]**

[a]*National Research Council Canada, Saskatoon, Canada*
[b]*Agriculture and Agri-Food Canada, Saskatoon, Canada*

Sateesh.Kagale@nrc-cnrc.gc.ca

Transfer of high value target loci from genetically diverse wild relatives to adapted elite varieties is seen as a key to the future of wheat breeding. Efforts to modulate homoeologous recombination between the chromosomes of alien donor and those of cultivated wheat via mutating the *Ph1* (*Pairing homoeologous 1*) locus, chemical treatment or changes in environmental conditions are currently underway. However, the fast progress of such projects is hindered by the lack of a rapid screening method for monitoring the impact of modulation of homoeologous recombination in polyploid crops, such as *Brassica napus* and wheat. Currently used cytogenetic methods for assessing recombination frequency are cumbersome and time consuming. The main objective of this study is to establish an easy and efficient method for monitoring the impact of modulation of recombination in plants. We have devised a strategy to assess homoeologous recombination frequency in an F_1 plant which leverages the combined advantages of single cell whole genome sequencing technology and the relative ease of enrichment of nuclei from pollens. Haploid pollen grain nuclei from an F_1 plant are ideal material to quickly assess homoeologous recombination frequencies as it is relatively easy to isolate thousands of pollen nuclei carrying segregating genotypes. Our method involves DNA isolation from single pollen nuclei derived from F_1 progenies using the Fluidigm C1 single cell auto prep system which offers a simplified single cell isolation and DNA extraction workflow. Subsequent sequencing of DNA and genotyping of multiple segregating nuclei from pollens facilitates assessment of the frequency of homoeologous recombination. The Fluidigm's C1 based single cell sequencing method works well for isolation of DNA from *B. napus* pollen nuclei. *B. napus* pollen nuclei were sorted successfully in individual Integrated Fluidic Circuit (IFC) wells. The nuclei capture frequency was about 40%. Bioanalyzer traces showed an enrichment of amplified DNA fragments at ~10 kb from the IFC wells in which pollen nuclei were captured. Successful PCR amplification of two well characterized *B. napus* genes further confirmed that the DNA isolated was derived from pollen nuclei. We are currently standardising wheat pollen nuclei cell capture and genome amplification. Future work will consist of the optimization of pollen nuclei capture frequency and confirming uniform coverage of whole genome amplification through sequencing.

The gene machine: Exploiting TILLING populations in a forward-genetics fashion

Riccardo Bovina[a], Sara G. Milner[b], Martin Mascher[b], Nils Stein[b], Agata Daszkowska[c], Iwona Szarejko[c], **Roberto Tuberosa[a]** and Silvio Salvi[a]

[a] *Department of Agronomic sciences (DipSA) - University of Bologna, Bologna, Italy*
[b] *Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany*
[c] *Department of Genetics - University of Silesia, Katowice, Poland*

roberto.tuberosa@unibo.it

Searching for mutations responsible for phenotypes-of-interest in a forward-genetics fashion was typically considered a multistep and challenging process. The recent advent of highly efficient mapping and next-generation-sequencing approaches considerably facilitates this procedure. For this purpose, we screened a barley mutagenized population (cv. Morex background, 3,071 M5 mutant families) to identify root morphology alterations. Four randomly chosen mutants were out-crossed to generate recombinant populations and to confirm their Mendelian inheritance. To speed-up the mapping procedure an SNP array (Illumina9K)-based analysis was applied on bulked recombinants allowing the fast and cheap identification of the target interval. A following exome-capture mapping-by-sequencing approach of the mutant lines was applied to link the observed mutant phenotype with the underlying gene. A candidate gene for a short-root mutant was identified by analyzing mutant allele frequency at called variants in the target region. The missense mutation, located in a conserved domain, is predicted to affect severely the protein function (SIFT score 0.01). Recently, we have performed a TILLING analyses (approx. 3,000 mutants of the cv. Sebastian) in order to identify alternative alleles affecting the candidate gene function. This preliminary results indicate our population as a useful resource for functional genomics and gene cloning in barley.

A R-based integrated pipeline for genomic and application to a wheat breeding programme

Gilles Charmet[a], Van-Giang Tran[a], Delphine Ly[a] and Jerome Auzanneau[b]

[a] *INRA-Université Clermont II UMR1095 GDEC, Clermont-Ferrand, France*
[b] *Agri-Obtentions, La Minière, France*

gilles.charmet@clermont.inra.fr

Since its introduction by Meuwissen et al, genomic selection (GS) has become a very powerful tool in plant breeding, with expected advantages over phenotyping selection relying on increasing selection intensity or decreasing the length of selection cycle (when technically and economically feasible). However, to keep GS as efficient as phenotypic selection, the accuracy of the genomic index should compete with that of phenotypic selection, i.e. heritability. In the framework of the French flagship project BREEDWHEAT, we developed an integrated pipeline based on R language (R development Core Team 20011), called BWGS (BreedWheat Genomic Selection pipeline). BWGS comprises three modules: 1) dimension reduction, by reducing the number of markers and/or training individuals, 2) missing data imputation and 3) Genomic estimation of Beeding Values (GEBV) with a choice among 11 parametric and non-parametric methods.
We explored the potential use of BWGS pipeline to estimate GEBV of yield and quality traits in bread wheat training population. A set of 760 breeding lines was genotyped with the BREEDWHEAT 423 K SNP chip, giving circa 150,000 polymorphic markers after quality control, with very few (0.5%) missing data. Phenotypic data came from historical breeding trials carried out from 2000 to 2014 in a multisite network in France. On average, every genotype was evaluated during 2.3 years, in 5.2 locations and 2 management systems (intensive vs low input) and 2 replicates per year*site combination. Mixed model BLUP of random genotype effects (i.e. corrected from environmental effects) were used as phenotypic traits to be predicted by molecular markers. The 11 prediction methods gave GEBV with very close accuracy (trough cross-validation), although significant differences were observed, with methods known to account for non-additive affects (e.g. EGBLUP or RKHS) giving slightly more accurate results. On this population, 5,000 to 10,000 randomly sampled markers seem enough to nearly achieve the highest accuracy, and imputation works very well, up to 40% generated missing data. As expected, the actual GEBV accuracies were very high (>0.6) for highly heritable traits such as pentosane viscosity of Fusarium scores, fairly high (# 0.5) for yield and protein content and moderate (# 0.3) for breadmaking scores, which were available on a smaller subset of the 760 lines). These results are encouraging for a successful use of genomic prediction as indirect selection criteria in wheat breeding programmes.
A zip file of the BGWS R-pipeline is available on request.

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Multiparameter quantification and metabolomics in plant science and breeding: NMR based test systems

Christoph Dotzer, Michael Rettig and **Roland Geyer**

numares AG, Regensburg, Germany

roland.geyer@numares.com

Analytical methods using information from the metabolome provide powerful alternatives to genetic methods, e.g. for poly genic traits, questions mainly triggered by genome by environment interactions or as systemic marker for abiotic stress. Furthermore, elite selection in breeding programs focussing on the concentration of valuable components or the determination of chemotypes are much more efficient when analysing every single plant.
Although several analytical methods are considered as gold standard, there are in every case certain drawbacks. These might be the low throughput when quantifying key components or the lacking reproducibility between subsequent sets of samples or different harvests.
numares provides a new and innovative approach based on nuclear magnetic resonance (NMR) spectroscopy to close this gap, to complement existing methods and to provide access to so far unused analytical possibilities. NMR is widely used for specific scientific studies since decades. However, up to now it is hardly found in routine laboratories. To enable the use of NMR in analytical routine, numares technology embeds classical NMR in a highly standardized and automated profiling.periphery and software solution. Thereby, the advantages of NMR, to be quantitative by nature, the universal and unbiased detection system and the broad dynamic range are combined with high throughput and efficiency.
The basic system can be used as research tool focussing on new questions and aiming at the joint development of customized tests. In addition, it was successfully used in several projects resulting in ready-to use test modules. These comprise the determination of total sesquiterpene lactone contents as well as the relative ratios of two groups of active sesquiterpene lactones in arnica, the quantification of inulin and polyisoprene in dandelion roots, quantification of carvacrol, thymol and thymochinone in oregano or the quantification of steviosid, rebaudiosid A, B and C in stevia leaves. Furthermore, up to 100 aqueous fermentation samples can be analyzed per day including the quantification of 70 substances in various combinations.
Beside targeted quantification of key components it is possible to use all signals provided in the spectra, representing up to several hundred ingredients. This enables a fast and reliable comparison of new samples with reference/master spectra for identification of similarities and differences or to identify fakes/adulterations. It is also feasible to determine metabolic profiles that allow for the classification of samples into groups (polygenic traits, origin, purity, production process …). numares proved for example the identity and purity of algal extract fractions after separation, ensuring the same extract composition in every production batch. Accompanying a hybrid rye breeding program the classification of hybrids in heterotic groups was realized.
In summary, the presented analytical system provides a powerful tool that is able to complement existing analytical methods focusing on automated multiparameter quantification in high-throughput and metabolic profiling.

mppR: an R-package for QTL mapping in multi-parent populations

Vincent Garin, Marcos Malosetti, Fred van Eeuwijk

Plant Sciences Group/Biometris, Wageningen University, The Netherlands

vincent.garin@wur.nl

Multi-parent populations (MPPs) represent an interesting resource to study plants genetics. MppR is an R-package dedicated to the joint QTL mapping of lines coming from different crosses with sharing of parents. Any type of population composed of more than one cross like nested association mapping (NAM) or and incomplete factorial design can be analysed. MppR allows to perform all steps of a QTL detection: data pre-processing, significance threshold determination, cofactors selection (SIM, stepwise regression), multi-QTL model search (CIM, iQTLm), cross-validation, genetic effect estimation and visualisation of the results. Different QTL detection models are proposed. They can be described according to two characteristics: the type of QTL effect assumed and the form of the variance covariance structure for the polygenic effect (VCOV).

QTL effects

Four type of QTL effect are possible. The assumption behind these models can be classified from the most specific to the most general. The cross-effect model assumes that allelic effects are different in each cross whereas the parental model assumes one effect per parental line that is independent of the genetic background. A third option allows to group parental alleles in ancestral cluster and assumes one effect per ancestral group. Finally, it is possible to use a bi-allelic model with effects attached to SNP marker alleles. Models can be built using a single type of QTL effect. A solution based on stepwise regression allowing each QTL position to have its own type of effect is also possible.

VCOV

Models can also vary according to the assumption about the variance covariance structure for the polygenic effect. The most basic model assumes a single polygenic variance. A second option uses cross-specific polygenic variances. A third option imposes a kinship relations on the polygenic terms. The final option consists of combining kinship with a cross-specific polygenic term.

MppR is a package covering a wide range of models and procedure for MPP QTL detection under different assumptions within a mixed model framework.

Application of biotechnology methods in plant breeding in Latvia

Dace Grauda[a], Nadežda Ļisina[a], Kārlis Žagata[a], Lita Lapiņa[a], Andra Miķelsone[a], Gints Lanka[a], Reinis Ornicāns[a], Karīna Latiševa[a], Aija Rebāne[b], Biruta Jansone[b], Sarmite Rancane[b], Aldis Jansons[b], Vija Srazdiņa[c], Inga Stafecka[c] and Isaak Rashal[a]

[a]*Institute of Biology, University of Latvia, Latvia*
[b]*Research Institute of Agriculture, Latvia University of Agriculture, Latvia*
[c]*Institute of Agricultural Resources and Economics, Research Centre Stende, Latvian University of Agriculture, Latvia*

dace.grauda@lu.lv

Production of doubled haploid (DH) wheat, barley and triticale plants by anther culture method is an important biotechnological approach, which enables significantly shortening breeding process. The best protocols for producing DH lines by anther culture from barley, spring and winter wheat and triticale hybrids from parents with unknown androgenic response were elaborated. The cold pre-treatment was applied for all used crops. The positive influence on embryogenesis of cupper (2.5 mg/l $CuSO_4$ x $5H_2O$) adding in induction medium was found. Two *in vitro* (anther and callus cultures) methods for obtaining flax breeding initial material were applied. Androgenic response of used hybrids was depending on genotype and growing conditions. Regeneration was observed only from diploid callus. iPBS retrotransposons based method of DNA fingerprinting was applied to find out genetic variability of regenerated plants caused by somaclonal variation. Flax rust resistance alleles at *L* locus were detected by molecular markers in the breeding initial material: alleles *L2*, *L6* or *L9* were found. The flow cytometry method for detection of flax pathogen's was developed. Cloning and cultivating methods of Latvian hemp breeding material and sex identification of female plants using the molecular markers were performed for enhancement of hemp breeding. There was found that the best medium for seeds shooting *in vitro* is medium with a half of MS medium salts, but for plant cultivation the best was MS medium with activated carbon. Side shoots for cloning were used. For the early sex identification of hemp plants this is an acceptable method, based on the PCR amplification of a male-specific SCAR marker. Last years in the red clover breeding program more attention was paid to creating tetraploid varieties (4n, 28 chromosomes), which are characterized by higher biomass and potential disease resistance. Methods were developed for enhanced obtaininging of tetraploid red clover breeding source material. *In vitro* methods for plantlets chromosome doubling by colchicine (0.2 % solution in water, 5h) and *in vitro* cloning of the tetraploid plantlets were elaborated. The BD FACSJazz cell sorter with the flow cytometer function was used for determination of ploidy level. Cells with different ploidy level were found after plantlets incubation in colchicine. Red clover plantlets with most than 60% of cells with ploidy 4n were used for cloning. The influence of genotype was observed on tetraploid cell development and plantlets surviving after colchicine treatment. Plantlets with well-developed roots in 2-3 leaves stage were planted in soil and grown in a greenhouse about a month, then replanted in the soil in field conditions and grown till the maturity. About 60% plants in the next generation give stable red clover tetraploid lines.

Expression of a variety of recombinant proteins in plants

Mokhtar Jalali-Javaran[a], Malihe Mirzaee[a], Ahmad Moieni[a], Sirous Zeinali[b], Mahdi Behdani[b], Masoud Shams-Bakhsh[a], Mostafa Modarresi[a,] Mehdi Mohebodini[a], Vahid Jalali Javaran, Houshang Alizadeh, Heydar Saifi Nabi Abad[a], Hossein Khosravi[a], Maryam Abdolinasab[a], Saba Dymyad[a], Nazanin Taheri Javan[a], Shahla Razmi[a], Babak Latif[a], Hamid Azhdari[a], Mojgan Soleimanizadeh[a]

[a] *Department of Plant Breeding & Biotechnology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran*
[b] *Biotechnology Research Center, Biotechnology Department, Venom & Biotherapeutics Molecules Lab., Pasteur Institute of Iran, Tehran, Iran*

m_jalali@modares.ac.ir

By using molecular farming, plants are used as a bio-reactor for producing low cost recombinant proteins. Over the last 15 years, different recombinant proteins with exceed of 100 types are produced in transgenic plants. Plants have many advantages in compare with other expression systems, especially in economic, safety, operations and production aspects. During last ten years of research in the field of molecular farming in Tarbiat Modarres University, different kinds of recombinant proteins such as VHH single domain antibody in tobacco and Canola and Tissue plasminogen activator (*tPA*) protein in tobacco, cucumber and potato were expressed. The human gamma interferon in Canola and tobacco, and pro-insulin in Lettuce and Strawberry were produced. Transplastomic plants have been considered because the plant's plastid genome is highly polyploid, the transformation of chloroplasts permits the introduction of thousands of copies of foreign genes per plant cell and generates extraordinarily high levels of recombinant protein. Also, useful projects are executed in the field of transplastomic plants in order to express the pro-insulin, human gamma interferon and Tissue plasminogen activator (*tPA*) genes in the chloroplast successfully. In this investigation expression and the enzymatic activity of tPA in different compartments by RT-PCR, ELISA and gelatin zymography was confirmed.

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Refined scaffold construction using PacBio sequences and LMP libraries of Korean pear (*Pyrus pyrifollia*)

Jung Sun Kim , Mi-Sook Seo , Kyoung Ah Lim, So Youn Won, Sangho Kang, Seong-Han Sohn

Department of Agricultural Biotechnology, National Institute of Agricultural Sciences, RDA, JeonJu, 54874, Republic of Korea

jsnkim@korea.kr

Pear is one of the most important temperature fruit species after grape and apple, belonging to th**e** subfamily Pomoideae in the family Rosaceae. The cultivated pear (n=17) has been growing since antiquity in both Europe and China. In Korea, three basic species, *P. pyrifolia*, *P. ussuriensis*, and *P. fauriei*, are known as native. We selected 'Wonwhang' (*P. pyrifolia*) for whole genome sequencing and could estimate a genome size of 535Mb by K-value 71. Long sequencing reads not only allow assembling and closing larger genomes including many plant and animal species, they also enable resolving complex repeats of extreme GC regions. We decided to sequence *P. pyrifolia* using PacBio P6-C4 chemistry and produced around 38Gb sequences with 40 SMART cells. Using the Celera Assembler PBcR pipeline with PacBio read self-correction and FALCON assembly, there were 6,463 contigs, 195Kb of N50 covering 582Mb. HaploMerger2 was used as assembly program to long-read and short-read sequences and we assembled a total genome size of 510Mb. Each LMP library was sequenced with 5Kb, 10Kb, and 15Kb and we finally obtained 2221 scaffolds, 649Kb of N50 and 516Mb assembled genome (96%) of 'Wonwhang' (*P. pyrifolia*).

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Plant varieties – “frozen” or plastic

Līga Lepse[a], Dainis Ruņģis[b] and Guntra Aistara[c]

[a] *Institute of Horticulture, Latvia University of Agriculture, Latvia*
[b] *Genetic Resource Centre, Latvian State Forest Research Institute “Silava”, Latvia*
[c] *Central European University, Hungary*

liga.lepse@puresdis.lv

Over previous decades, the topic of plant genetic resources (PGR) has been discussed in many contexts. Several polices have been developed and applied, differing between countries and over time, but one concept has been constant – cultivated plant varieties of local origin and landraces are considered as local genetic resources. According to some publications and investigations, plant domestication started 13 000 years ago and today provides most of our food. Domestication has been continuing over centuries and has finally brought us to the situation where we are now – with many lost or endangered species, varieties and cultivars. After signing the Rio de Janeiro Convention on Biological Diversity (CBD) in 1992, activities in PGR conservation and maintenance were initiated in many countries and intensified in more `experienced` countries. In some contexts, these activities were contrary to commercial cultivar breeding and there was a period when practical PGR work and legislation was adapting to this new situation with unclear ‘playing rules’. Commercial breeding companies with advanced breeding technologies and their pre-breeding strategies (often based on using PGRs together with biotechnology tools demanding high inputs) insisted on strong protective legislation, while local landrace growers, collectors and “seed savers” growing unprotected cultivars/populations insisted on less restrictive seed growing/marketing rules. These differences have been debated in the context of the review of EU seed regulations over several years. In Latvia, 2012 was the year of the “tomato rebellion” which brought both sides to the negotiating table. The outcome of these discussions was an understanding of the duality of the situation where two different views on plant varieties can be distinguished:

A variety in the context of conventional agriculture is a genotype which is `frozen` at one particular moment of development and maintained in this state by strong, legally approved seed propagation rules.

A variety in the context of landraces/on-farm/*in situ* conservation and use is an ecologically flexible [plastic] genotype/ population that can be variable in time and space, but that exhibits the original characteristic traits of the variety, that may change slightly during less regulated seed production processes. Seed propagation is performed in a less restricted manner. In order to maintain the adaptive potential of genetic resources for the future, particularly in the context of changing climatic conditions and commercialisation of breeding programmes, this approach has to be continued and legally institutionalized in Europe to successfully ensure the conservation and use of landrace/*on-farm*/*in situ* genetic resources.

Association of grain yield, drought tolerance indices and proline accumulation in selection for drought stress tolerance in soybean

Obed J. Mwenye[a], Leon van Rensburg[b] and **Rouxléne van der Merwe[a]**

[a]Department of Plant Sciences, University of the Free State, Bloemfontein, South Africa
[b]Department of Soil, Crop and Climate Sciences, University of the Free State, Bloemfontein, South Africa

vandermerwer@ufs.ac.za

Drought tolerance is a complex quantitative trait involving interactions of many metabolic pathways. In the absence of an understanding of the special mechanisms of tolerance the quantification of drought tolerance should be based on grain yield under dry conditions. Breeding for drought tolerance is complicated by the lack of fast, reproducible screening techniques and the inability to routinely create defined and repeatable water-limited-induced-stress (WLIS) conditions. Several yield-based tolerance indices, based on mathematical relationships between yield under non-stress conditions, have been proposed to characterise the behaviour of genotypes in stress and non-stress conditions, and to screen drought tolerant genotypes. In addition to yield-based drought tolerance indices proline accumulation in genotypes under stress conditions can be used as a further selection criterion for drought tolerance. The objectives of this study were 1) to determine associations among yield potential, the tolerance indices and proline accumulation under WLIS and non-stress conditions and 2) to compare the capacity of the different indices to identify genotypes having high yield under both WLIS and non-stress conditions. A replicated glasshouse experiment was planted in the weighing lysimeter unit at the University of the Free State during 2014 and 2015. The trial was laid out in a randomised complete block design with two factors (genotypes and water treatments) and three replications. Four genotypes were evaluated for seed yield (g plant^{-1}) under WLIS (Y_S) and non-stress (Y_P) conditions. Drought tolerance indices including mean productivity (MP), stress susceptibility index (SSI), stress tolerance (TOL), stress tolerance index (STI), geometric mean productivity (GMP), yield index (YI) and yield stability index (YSI) were determined. Significant and positive associations were observed among seed yield potential, proline accumulation and the drought tolerance indices. The strong positive correlations of MP, GMP and STI with Y_P and Y_S indicated that these indices were more effective in identifying high yielding genotypes under WLIS and non-stress conditions. Principal component analysis confirmed the data structure and relations of yield, proline and the drought tolerant indices. PC-1 was related to grain yield (Y_P), GMP, MP and TOL while PC-2 was mostly explained by TOL, Y_S, GMP and MP. The most prominent associations were among TOL, proline under stress and relative change in proline, and between STI and MP. The drought tolerance indices were effective in separating genotypes in terms of yield potential under both WLIS and non-stress conditions. No specific index can be used exclusively to discriminate drought tolerant genotypes from susceptible ones. The correlation matrix and PCA analysis gave complementary information and such knowledge could assist breeders in making selection decisions. Future studies should include more genotypes and indices need to be evaluated under field conditions.

Germplasm exchange and use in Italian national research institutions

Maria A. Palombi[a], Petra Engel[a] and Flavio R. De Salvador[a]

[a]*Fruit Tree Research Centre, Council for Agricultural Research and Economics (CREA), Rome, Italy*

mariaantonietta.palombi@crea.gov.it

Maintenance, management and strategic visions of germplasm collections have received increased attention during the last years, due to different practical, cultural and political reasons. The FAO International Treaty on Plant Genetic Resources for Food and Agriculture (PGRFA), entered into force in 2004, promotes the collection, conservation and sustainable use of genetic resources, and many countries have national laws or action plans in place to implement these targets. Awareness of historical and cultural importance of PGRFA, at national and regional level, further encourages the exploration and identification of these resources, including their wild relatives. At the same time, increased flow of germplasm from one genebank to another, nationally and internationally, as well as international collaboration programmes on PGRFA conservation and use have stimulated the elaboration of codes of conduct on germplasm management, characterization and exchange practices. An analysis of conservation, utilization and exchange practices is useful also in view of the Nagoya Protocol on Access and Benefit Sharing of Genetic Resources of the CBD, recently entered into force, in order to clearly define the position and significance of PGRFA in the wider context of genetic resources and related exchange regimes. Since 2004, The Italian Ministry of Agriculture, Food and Forestry Policies (MiPAAF) finances a targeted project on the implementation of the FAO International Treaty, currently involving, amongst other participants, 29 research institutions of the national Council for Agricultural Research and Economics (CREA). These institutions hold approximately 45.000 accessions belonging to more than 250 genera and over 800 species of agriculturally important crop categories (cereals, vegetables, fruit, forages, medicinal and aromatic species, ornamentals, citrus, olive, grape, as well as forest species). Exchange of material, both supplying and receiving, are common operations in these institutions, for different purposes. The present work analyzes the quantity and nature of germplasm entered in the collections, motivations and modalities of germplasm flow to and from the institutions, after 10 years of implementation of the FAO Treaty. Strategic priorities and specific purposes for germplasm acquisition are presented and discussed, as well as motivations for utilization of exchanged material. Main aspects are: a) number of accessions entered into the collections since the entry into force of the FAO Treaty, targeted implementation of collections after gap analysis, b) specific collection of local/national germplasm, c) agricultural/alimentary or other interest, d) research and breeding purposes, e) other motivations for exchange and delivery (safety duplication, educational purposes, supply to small farmers, home or hobby gardeners, etc). It also assesses the type of exchange partners (private/public) and their affiliations (research institutions, breeding companies, botanic gardens, private collections, foundations etc). In addition, we will analyze the kind of arrangements for exchange of material being utilized (sMTA of the Treaty, other legally binding arrangements, institutional or individual agreements, etc). The purpose of this study is to present insights in Italy's national and international PGRFA conservation and exchange provisions and its contribution to future material utilization regimes.

High-throughput screening tools for identification of traits contributing to salinity tolerance in *Arabidopsis thaliana*

Klára Panzarová[1], Mariam Awlia[2], Ariana Nigro[3], Jiří Fajkus[1], Magdalena M. Julkowska[2], Mark Tester[2] and Martin Trtílek[1]

[1]*PSI (Photon Systems Instruments), Drásov, Czech Republic*
[2]*Center for Desert Agriculture, KAUST, Saudi Arabia*
[3]*Institute of Plant Biology, University of Zürich, Switzerland*

panzarova@psi.cz

Advances in genome sequencing need to be accompanied by reproducible and efficient high-throughput phenotyping approaches to facilitate the discovery of new genes underlying enhanced plant performance. Soil salinity poses a great challenge for modern-day agriculture with most breeding efforts focusing on salinity tolerance using shoot-ion exclusion as the key phenotype. Upon the first exposure of the roots to salt stress, plants suffer a rapid growth reduction which is occurring prior to the accumulation of ions to toxic concentrations in the shoots. During this early phase, photosynthetic activity of the plants decreases and plant growth is reduced, which includes slower leaf emergence and a small growth size.

To enhance our understanding of the early plant responses to salinity, we designed an experimental protocol based on using high-throughput phenotyping system developed at Photon Systems Instruments (PSI, Czech Republic). Plant growth, morphology and photosynthetic activity determine overall plant performance collectively. Therefore we established a methodology based on automated integrative analysis of photosynthetic performance, growth analysis and colour index analysis at the onset and during early phase of salinity stress responses of nine *Arabidopsis* accessions cultivated under controlled conditions in soil. Here we show that imposition of plants to mild salt concentration of 100 mM NaCl in the soil-water, rapidly affected photosystem II operating efficiency, reduced growth dynamics and dynamically changed colour index of plants at different stages of stress response.

Selected photosynthetic performance- related parameters were clustered with relative plant growth rates into traits corresponding to early and late changes in response to salt stress. Further the natural variation in the quantified traits was characterized. Our analysis enabled the categorization of changes in phenotypic traits upon imposition of stress, which can be used as markers for early or late salt stress responses, and provide insights into the underlying processes of plant salinity tolerance.

This work presents an integrative approach, which allows simultaneous analysis of different phenotypic traits. The outlined phenotyping protocol in combination with intensively genotyped genetic resources is expected to improve our knowledge of plant performance and stress response dynamics, and lead to the identification of desirable target genes contributing to crop improvement.

Information system GRIN Czech of plant genetic resources in the Czech Republic

Ludmila Papoušková and Vojtěch Holubec

Crop Research Institute, Gene Bank, Czech Republic

papouskova@vurv.cz

The study and conservation of plant genetic resources (PGR) in the Czech Republic has a long tradition. Since 1993, the efforts on PGR have been concentrated within the National Programme on Plant Genetic Resources. This project covered all of the basic activities on plant genetic resources; specifically gathering (including collecting expeditions), documentation, characterization, evaluation, conservation of PGR and also services to users of PGR. 12 institutions hold more than 53 thousand accessions (belong to 978 species); 18 % of which are vegetatively reproduced species. The Crop Research Institute (CRI), Prague, has overall responsibility for coordinating the Programme, runs the national information system on PGR (GRIN Czech) and provides long-term storage for all seed-propagated species. All of the Czech collections are fully documented with passport data and characterisation and evaluation data (based on National Descriptor Lists) is available for 65 % of the accessions. Each year about 2 500 samples of PGR are provided to users.

Documentation of plant genetic resources, as well as services to the curators of collections, gene bank managers and users of the genetic resources, had been provided by system EVIGEZ for 20 years but in 2015 was replaced by system GRIN Czech. The system was provided by the USDA Agricultural Research Service and it is a compatible version of GRIN Global, developed jointly by the USDA Agricultural Research Service, Bioversity International, and the Global Crop Diversity Trust. It consists from many parts: passport and provenance (Accession ID, Taxonomy, Accession name, Origin, Material Type, Maintained By, Availability, Intellectual Property, Standard Material Transfer Agreement Status), germplasm inventory (e.g. number of seeds in storage), requests for germplasm and order fulfilment and also enables to record characterization/evaluation data, including genetic marker observations and etc.

Passport data of all PGR from Czech collections is now available in the system GRIN Czech together with characterization and evaluation data of more than 65 % of the accessions. This value is increasing with the systematic evaluation of the collections because this evaluation substantially enhances the value of collections for breeders and other users. For most crops there are evaluated 20–40 characteristics. All information about the collections is available on this website: https://grinczech.vurv.cz/gringlobal/search.aspx.

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Hyperspectral image processing using visual programming high throughput plant analysis on field scale

Stefan Paulus[a], Tino Dornbusch[a], Kevin Nagel[a] and Marcus Jansen[a]

[a] *LemnaTec GmbH, Germany*

stefan.paulus@lemnatec.de

Hyperspectral imaging has been extensively used for vegetation assessments and increasingly for plant phenotyping during the last years. In particular, vegetation indices for water stress responses or symptoms of plant diseases were established. Moreover, data mining approaches allow in-depth analysis of plant stress responses. With the rise of digital field phenotyping, hyperspectral image data at field scale is available and huge datasets in the size of many gigabytes of data have to be analysed. This can only be done by close interaction of experts in plant- and data science using an adapted analysis software.

The image processing toolbox of LemnaTec GmbH is a general purpose computer vision software for the automated analysis of huge amounts of different kinds of sensor data. It has widely been used for the extraction of plant parameters like size, colour, and shape out of 2D RGB images of different types of plants. Furthermore it is able to process hundreds of images in high throughput using an objective and comparable processing algorithm. Segmentation of single plant parts is possible for plant images on different scales from microtiter plates, petri dishes, and single plants in the greenhouse or on field scale. Once created parameterisation pipelines can be easily adapted to different plant species. One main feature is its simple and intuitive visual programming interface to enable users to translate their ideas into working image processing algorithms.

The use of hyperspectral image processing has been integrated into the software and an efficient parameterization of plants in the hyperspectral space is possible. An application scenario using this software and hyperspectral data from a field experiment was carried out as a pilot study. Vegetation indices were extracted out of hyperspectral data cubes and combined to a field map showing different plots with their corresponding vegetation indices. We show that nitrogen fertilisation related vegetation indices characteristics were highly correlated with the nitrogen level applied to the single plots.

The image analysis software LemnaGrid (LemnaTec GmbH, Aachen) provides a professional tool that enables the intuitive connection of different image processing algorithms. It is adaptable for different plant types and on different scales from the microscopic scale to the field scale and enables the processing of huge amounts of data in high throughput.

Harmonization of resistance tests to diseases for DUS testing – Harmores 2

Sophie Perrot[a], Mathilde Buffard[a], Olivier Herbert[a], Sandrine Houdault[a] and Valérie Grimault[a]

[a] *Pathology laboratory, GEVES, France*

sophie.perrot@geves.fr

For variety registration and protection, DUS (distinctness, uniformity, stability) tests are carried out by examination offices and include evaluation of resistance of varieties to different pests, following CPVO and UPOV protocols. In order to avoid discrepancies between resistance declarations from breeders and official tests and to have equivalent results of resistance testing in the different examination offices, harmonization of resistance tests protocols was needed. Harmores 2, a three year project co-founded by CPVO, was set up and managed by GEVES (FR) in collaboration with six examination offices: Bundessortenamt (D), INIA (SP), Naktuinbouw (NL), NEBIH (HU), SASA (UK), UKZUZ (CZ) and affiliates JKI (D) and UPOL (CZ), and five ESA members (European Seed Association) representing the seed industry. The aim of this project was to harmonize, at the European level, resistance tests to seven vegetable diseases *Bremia lactucae*/lettuce, *Fusarium oxysporum* f. sp. *pisi* race 1/pea, *Ascochyta pisi* race C/pea, TMV:0/pepper- PMMoV:1.2/pepper - PMMoV:1.2.3/pepper- -PVY:0/pepper.

For each of them, the detailed objectives were the definition of reference material (isolates, controls and differentials), test conditions, notation scales and decision rules. Ring tests were organized to validate the reproducibility and repeatability of the developed tests and propose robust harmonized protocols.

In the framework of this project, GEVES organized in 2014 two workshops on lettuce/*Bremia* (including 8 seeds companies) and pea/*Fusarium*. National offices of seven European countries and ESA representatives participated. These workshops have allowed establishing harmonized notation scale and decision rules for both diseases.

For each host/pathogen combination, reference material and test conditions were selected based on the availability of reference material (providers and maintainers of isolates were defined and indicated in CPVO protocols) and the capacity of test in the different laboratories. Seven updated robust protocols were proposed to CPVO, allowing consistent results between examination offices and breeders. A proposition of revision of the Lettuce UPOV guideline was done.

A future Harmores 3 project is now proposed on another set of seven host/race/pathogen combinations selected in collaboration with partners of the project and focusing on intermediate resistance.

Optimized and cost-efficient genotyping arrays for plant breeding

Joerg Plieske[a], Jana Lemm[a], Hartmut Luerssen[a], Eva-Maria Graner[a], Andreas Polley[a] and **Martin Ganal**[a]

[a]*TraitGenetics GmbH, Stadt Seeland OT Gatersleben, Germany*

ganal@traitgenetics.de

Genotyping with many molecular markers is now an important tool for diversity analyses, genetic mapping and plant breeding. TraitGenetics has been involved in the development and characterization of many genotyping arrays for important field crops. Such first generation SNP arrays usually contain a relatively large number of markers that are difficult to score, are polymorphic only in a limited set of germplasm, are not creating additional information since they are in perfect LD with other markers on the array and are sometimes clustered in specific regions of the genome. This makes first generation arrays relatively expensive in applications for plant breeding where costs per sample are a crucial factor. In order to reduce costs, we have developed second generation SNP genotyping arrays based on genotyping data that have been collected during our own internal genotyping of breeding material and genetic resources. We present the procedures employed for the development of such arrays for hexaploid and tetraploid wheat, tetraploid and diploid Brassica species and maize. These new arrays contain only high quality markers that are mostly haplotype-specific, are evenly distributed over the genetic map and contain markers for known genes which are associated with specific traits of relevance for breeding. Such arrays lower genotyping costs to a level that makes genotyping of large sample numbers (many thousands) feasible in the routine plant breeding process including large scale genomic selection.

Metabolic profiles and viral charge genome wide mapping in Arabidopsis thaliana in response to TuMV (Turnip mosaic virus) in a natural environment

Bernadette Rubio[a], Patrick Cosson[a], Mélodie Caballero[a], Frédéric Revers[b], Yves Gibon[a], Fabrice Roux[c] and Valérie Schurdi-Levraud[a]

[a]*UMR Biologie du Fruit et Pathologie, INRA Université De Bordeaux, France*
[b]*UMR BIOGECO, INRA Université De Bordeaux, France*
[c]*Laboratoire des Interactions Plantes Micro-Organismes, CNRS INRA, Toulouse, France*

brubio@bordeaux.inra.fr

When studying the genetic basis of plant/virus interactions, qualitative or major resistance factors are often targeted and experiments are mostly conducted in controlled conditions. In a changing environment, a major challenge in plant breeding and evolutionary biology is now to identify the genetic and molecular bases for natural resistance/susceptibility variation to various stresses in plant species in a natural environment.
Therefore, the present project aims at identifying the biological and genetic basis of susceptibility of *Arabidopsis thaliana* to a plant virus in natural environmental conditions using the natural pathosystem *Arabidopsis thaliana*/*Turnip mosaic virus* (TuMV). *Arabidopsis thaliana* is a powerful model for studying local adaptation and for identifying genetic variants that associate with a quantitative trait. Among the genus *Potyvirus*, *Turnip mosaic virus* is probably the most widespread and damaging virus that infects brassicas worldwide.
In this study 324 genotypes of *Arabidopsis thaliana* are grown in a common garden. These genotypes represent a large genetic diversity of World and French populations. Plants are mechanically inoculated by the same standardized TuMV UK1 inoculum. Thirteen days after inoculation, viral accumulation is evaluated by a DAS ELISA in systemic parts of the plants. The same samples are analysed for primary metabolites content on the metabolomics platform of Bordeaux (INRA, UMR BFP, Bordeaux – France).
Viral charge and other traits related to disease were used in a genome wide mapping analysis. We thus identified some genetic determinants involved in *Arabidopsis thaliana* response to TuMV infection. This strategy allowed us to map loci previously known to be involved in plant/potyvirus interaction. We also highlighted *de novo* candidate loci. Some of them seem to be experiment dependent and clearly involved in responses in non-controlled conditions.
Metabolomics represents an important addition to the tools currently employed in genomic assisted selection. In that way, twelve primary metabolic profile analyses were performed. An increase of these primary metabolites was usually observed in response to virus infection. Moreover, a significant difference in primary metabolic content was found between resistant and susceptibility genotypes to TuMV. These main results suggest that primary metabolomics traits could be a marker of resistance/susceptibility in *A.thaliana*/TuMV interaction. The mapping of these traits is ongoing.

Comparing the light transmissibility of pollination bag materials

Hannah Senior[a] and **Daljit S. Virk**[b]

[a]*PBS International, Salter Road, Scarborough, UK*
[b]*School of Environment, Natural Resources and Geography (SENRGy), Bangor University, Bangor, Gwynedd, Wales, UK*

d.s.virk@bangor.ac.uk

Pollination bags are used by plant breeders and seed producers to maintain the genetic identity of hybrid seed by excluding foreign pollen and to maximise the quantity of healthy hybrid seeds. Yet often, little attention is paid to the type of pollination bags used. Different material traits determine micro-environment within the pollination bag. For instance light transmission, humidity, temperature and aeration all affect seed set and development. The light transmission properties have an important bearing on metabolism of the covered parts of a plant since different photosynthetic pigments of plants use different wavelengths and different wavelengths accomplish different growth and development processes. The ultraviolet light (10nm-400nm) in small amounts can have beneficial effects but overexposure to UV light is dangerous. 385 nm UV light promotes the accumulation of phenolic compounds, enhances antioxidant activity of plant extracts. Blue light (430nm-450nm) spectrum affects chlorophyll formation and photosynthesis processes. Green light (500nm-550nm) encourages vegetative growth and intense photosynthesis. Red light (640nm-680nm) affects phytochrome reversibility and is the most important for photosynthesis, flowering and fruiting regulation. Far red (730nm) wavelength is outside the photosynthetically active range, but may increase the temperature.

In the current study, the impact of materials choice on the light availability inside the pollination bag was explored by evaluating six materials of pollination bags for light penetration (%) of different wavelengths (nm) varying from 200 nm to 1000 nm at 0.5 nm intervals; that is, the visible spectrum including the range most relevant for plants. The materials were: Kraft paper, Orchard Wholesale white paper, Edor Skoglund Canvas, Tyvek, Cellulose and duraweb®. The light transmission was measured at three positions on the fabrics; vertical, horizontal and across which were the replicate blocks in the analysis of variance.

There were highly significant differences between materials with a wide difference in mean light transmission ranging from 3.94% for Tyvek to 86.63% for Cellulose. The mean per cent light transmission showed very distinct groupings which were: Lowest for Tyvek (3.94%); Low for Kraft Paper (15.78%) and Orchard Wholesale (18.40%); Medium for Edor Skoglund Canvas (23.42%); Medium-High for duraweb® (28.61%); and Highest for Cellulose film (83.63%). The scatter of light transmission (%) against wavelength (nm) showed that Cellulose had the highest transmission at all wavelength from 300 to 1000 nm. This was followed by duraweb® fabric. All other fabrics had much lower transmission at all wavelengths. Tyvek was the worst and Kraft paper showed a rise of transmission after 700nm.

A DNA repository platform for germplasm collections

Michael Stierschneider[a] and **Eva M. Sehr**[a]

[a] *Health & Environment Dept., AIT Austrian Institute of Technology GmbH, Austria*

eva-maria.sehr@ait.ac.at

DNA banks or repository platforms for germplasm collections represent important facilities for the preparation and conservation of diverse genetic material over years and even over decades. Throughout the world, biodiversity is under serious threat from factors such as intensive agriculture, increased habitat fragmentation, climate change, and exposure to pollution and mass tourism. Furthermore, plant genetic resources of major and minor world crops are suffering genetic erosion, thus, activities are ongoing to preserve and characterize them. In this respect, DNA collections have become important resources in worldwide efforts to address the biodiversity crisis, manage the world's genetic resources and maximize their potential.

Therefore, the DNA bank at AIT Austrian Institute of Technology offers storage and access to quality assured material (genomic DNA, tissue) and data from various national and international research projects (e.g. EVOLTREE). Due to the broad availability of major germplasm collections for crops, the DNA bank at the AIT focused on forest trees as well as orphan crops. Over the last years we established specific protocols to extract high quality genomic DNA from leaves, needles, roots, buds, cambium and even processed wood and offer individual protocol development on demand. Facilities for high throughput genomic DNA extraction (liquid handling platforms), PCR and quality control are available. The core of the DNA bank represents a full automated storage unit for several thousand samples at -20°C. Barcoded sample tracking together with a self-established LIMS system [1] guarantee reliable data and sample management. Sample access is provided via the homepage www.dnabank.at, an integrated search interface called e-lab is available at www.evoltree.eu.

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National plant genetic resources in the Vilnius University Botanical Garden

Gitana Stukeniene and Audrius Skridaila

Vilnius University Botanical Garden, Lithuania

stukeniene@gmail.com

Nowadays, botanical gardens are seeking to play a key role in the conservation of plant diversity of the world. Many botanical gardens work together or in close collaboration with other research institutions in order to coordinate and target their conservation activities. The biggest treasure of any botanical garden is its plant collections. In terms of the size of these collections, the Vilnius University Botanical Garden in Kairenai (further – the Botanical Garden) is the largest in Lithuania and among the largest ones in the Baltic States. As of 2015, plants of over 10 000 taxa are grown in the Botanical Garden. They include 102 specimens listed in the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species. Moreover, 49 specimens are listed in the Lithuanian Red List; four had been confiscated by the Lithuanian Customs Service, which were on the CITIES (the Convention on International Trade in Endangered Species) list. Also, there are 1972 specimens listed in the Botanical Gardens Conservation International (BGCI) database, and 280 specimens are listed in the Lithuanian Ornamental Plant Genetic Resources database (104 ornamental plants, 34 trees or tree groups, 18 garden plants, 121 outdoor plants, and three species of native Lithuanian flora). Two other plant collections in the Botanical Garden have the status of Lithuanian Ornamental Plant Genetic Resources: first, the Lithuanian black currant (*Ribes nigrum* L.) and gooseberry (*Ribes uva-crispa* L.) collection, and, second, the J. and E. Tarvidas peony (*Paeonia lactiflora* Pall.) collection. Some special plant groups are grown in collection plots; however, most of them are grown in garden plots accessible to the public. In 2003, the Lithuanian Ornamental Plant Genetic Resources Coordination Center was established in the Botanical Garden. It coordinates the collection, evaluation and selection of several species, partly in the frame of the Lithuanian National Plant Genetic Resources Programme managed by the Ministry of Environment of the Republic of Lithuania. The plant species granted the status in the above-mentioned programme are described in a manner corresponding to the documents of Bioversity International (BI) requirements, and the data are recorded in the central database. In 2004, the European *Ribes* L. and *Rubus* L. Plant Genera Registration Center with a dedicated database were established in the Botanical Garden according to the agreement with the International Plant Genetic Resources Institute in Italy. For many years, the Botanical Garden has been part of the International Plant Exchange Net: it has been exchanging seeds with more than 300 partners in over 50 countries. Every year about 800 parcels of plant seeds are sent to research institutions worldwide and about the same amount of seeds is received in exchange.

Longevity of elite cultivars is a matter of conservation rather than maintenance breeding

Ioannis S. Tokatlidis

Department of Agricultural Development, Democritus University of Thrace, Greece

itokatl@agro.duth.gr

The longevity of elite cultivars is a serious concern for breeders due to time-consuming and costly endeavors to breed them. Therefore, the way the breeder-seed is treated for cultivar maintenance is of prime importance. In currently applied schemes, just rouging of plants obviously deviating from the mean type ('off-types') and those carrying seed-borne diseases is deemed adequate to secure breeder-seed uniformity and healthiness. Nevertheless, residual heterozygosity and molecular mechanisms that generate *de novo* variation as a response to environmental forces result in considerable intra-cultivar variation. An extensive review of the literature into investigations that have evidenced intra-cultivar genetic variation and those pertaining to the response to intra-cultivar selection indicate the necessity of a "breeder-seed conservation" technique via perpetual intra-cultivar breeding instead of the "breeder-seed maintenance" procedure. Hence, a "conservation breeding" strategy is presented, comprising two distinct processes. Both are based on single-plant selection under minimum interplant competition (widely apart plants to preclude plant-to-plant interference for inputs), for two major reasons. Firstly, minimum competition accelerates the limited intra-cultivar variation allowing stewardship of it properly. Secondly, minimum competition is necessary to cope with the negative relationship between competitive and yielding ability that exerts suspending effects on selection efficiency and recognition of superior genotypes. One "conservation breeding" process aims to secure breeder-seed devoid of harmful alterations: it is a periodical breeder-seed advancement ending up with *standard (prototype)* breeder-seed, part of which is kept as stock and the rest constitutes the threshold of the new breeder-seed reproduction round. The second is a continuous selection function to either replenish the breeder-seed or feed pre-basic seed to the multiplication rounds of certified seed. Within the framework of cultivar conservation, beyond uniformity and sanitary status, sustainable cultivar management would be attained through the exploitation of adaptive responses to ever-changing biotic and abiotic agro-ecosystems.

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Impedance flow cytometry - An ingenious method for pollen viability determination

Ruthger W van Zwieten[a], Grit Schade[a], Iris Heidmann[b], Marcel Ottiger[a] and Marco Di Berardino[a]

[a] *Amphasys AG, Switzerland*
[b] *Enza Zaden Research and Development B.V., The Netherlands*

ruthger.vanzwieten@amphasys.com

Analysis of pollen viability plays an important role in various aspects of plant breeding and plant production processes. Pollen viability is generally determined by various classical methods like staining techniques or *in vitro* germination assays. The disadvantage of the numerous staining techniques is that they have to be adapted per species and the resulting data do not always correlate with *in vitro* germination. Both, the current methods analysing pollen viability and germination are limited in the number of cells that can be analysed in a certain time frame and they are laborious in preparation and analysis. Here, we present a novel, label-free approach for the determination of pollen viability and maturation grade based on high-throughput single cell analysis by impedance flow cytometry (IFC). The technique is based on an improved Coulter counter which analyses individual pollen grains via a microfluidic chip and permits impedance measurements in a broad radiofrequency (RF) range (0.1 – 30 MHz), allowing detailed cell characterisations. We show that besides measuring pollen viability, also different stages during pollen development can be discerned. Using IFC, pollen from virtually any crop, vegetable or ornamental species can be assayed for viability.

Increasing the seed yield through better-quality pollination bags

Daljit S. Virk[a] and **Hannah Senior[b]**

[a]*School of Environment, Natural Resources and Geography (SENRGy),Bangor University, Bangor, Gwynedd, Wales, UK*
[b]*PBS International, Salter Road, Scarborough, UK*

hannah.senior@pbsinternational.com

Plant breeders and seed producers have a common goal of maximising the hybrid seed production and genetic identity of the hybrid without contamination from foreign pollen. This is often accomplished by deploying pollination bags. Such products are available in limited range in the open market; often bags designed for other purposes are used. The characteristics of fabric, plastic or paper used to manufacture the pollination bags determine the micro-climate within the bag in terms of humidity, temperature, aeration, and disease development, as well as the chances of the bag itself resisting damage due to water, wind or birds; all of these factors consequently affect the development of seed. Nonetheless, not much attention has been hitherto placed on testing the role of pollination bag choice in producing healthier seeds in higher quantity.

We have evidence from a number of experiments over several commercially significant crops that the choice of pollination bag influences hybrid seed production. We describe here five examples from a wide range of crop plants to illustrate the influence of pollination bags on the quantity and quality of hybrid seed harvest. The five studies are: one each from oil palm (*Elais guiniensis*), loblolly pine (*Pinus taeda*), *Miscanthus spp*., and two from sorghum (*Sorghum bicolor*). In all these studies nonwoven fabrics especially designed and selected for the specific plant breeding applications were found to be superior to the traditionally used bag types.

In oil palm, such bags produced 13% more seed than the alternative canvas and generically produced nonwoven bags, and did not show signs of contamination by pollinating weevils. In loblolly pine flower retention in June of conelets produced via controlled mass pollination was significantly greater in certain specifically-designed non-woven fabric than the equivalent traditional paper bags. In Miscanthus, bags made from a polyester material specifically designed for pollination control were found to result in 15% higher success rate of hybrid seed set versus the traditional bag type. Such bags were also found to be re-usable, slug resistant and withstand the overgrowth of panicles within them more successfully than paper bags. In two studies on different sorghum varieties (one in India, one in Brazil), bags specifically designed for this purpose were found to produce higher panicle weight and seed weight, and lower incidence of mold in the rainy season.

While interesting in themselves, these discoveries also have an economic implication for plant breeders. Often breeders describe how higher seed production of F_1 crosses or in early segregating generations of crosses can enhance breeders' capacity of yield testing of progenies, by avoiding repeating cycles. Reducing the frequency of repeats may have substantial benefits to the pace of a breeding programme. Furthermore in two of the studies economic analysis was also undertaken, and in both cases this suggests that spending more on a higher-performing pollination bag is more than offset by economic benefits elsewhere in the programme.

High throughput phenotyping of winter wheat canopy cover and plant height development in response to temperature

Kang Yu, Lukas Kronenberg, Norbert Kirchgessner, Achim Walter and Andreas Hund

Institute of Agricultural Sciences, ETH Zurich, Switzerland

kang.yu@usys.ethz.ch

Plant early vigor of winter wheat is often reflected by its early development of tillers, which is one of the most important indicators of crop yield. Moving from tillering stage into stem elongation inclusively, a relatively long vegetative phase is often required for high yielding winter wheat varieties. Within this critical phase, temperature is one of the main factors affecting early vigor and duration of stem elongation. However, it is difficult, in a fast and high throughput way, to keep track of the growth response of winter wheat to temperature using traditional, labor-costly methods. In this context, novel optical methods have been applied for high throughput phenotyping of plant traits, and they must be adapted to meet the practical needs of crop breeding in the field.

In the present study, a near infrared camera and a laser scanner were used to measure the winter wheat canopy cover (CC) and plant height (PH) development, respectively, during tillering and stem elongation stages. The main objective was to identify phenotypic variation of winter wheat growth in response to temperature, and finally we would attempt to link it to the genetic variation. A field experiment comprising more than 300 European winter wheat genotypes was conducted in two growing seasons at the ETH research station Lindau-Eschikon. Canopy cover and PH were measured at intervals of 3-4 days, partially with the aid of the ETH Field Imaging Platform (FIP). Growth rates based on CC and PH were calculated, and their responses to the temperature determined as the slopes (α) for linearly regressing the growth rates on temperature, i.e., $Y=\alpha X+\beta+e$. Results showed that daily growth rates differed markedly between measurement intervals, as well as varied among genotypes. By examining the growth in certain time spans of the vegetative phase, significant genotypic effects on the responses to temperature and vapor pressure deficit (VPD) were detected ($p < 0.001$). Heritability of the response to temperature was around 0.50 for both of the CC- and PH-based growth rates, whereas it was 0.90 for CC-based growth rate observed within a subset of 30 Swiss cultivars with more replications. The varying heritability suggests the complexity of environmental effects and multiple factors affecting the growth response across years. Genome wide association study (GWAS) revealed pronounced marker-trait-associations (MTAs) and quantitative trait loci (QTLs) for the growth response to temperature changes, which might also suggest new insights into the links between vernalization response and important crop traits. The results highlight that the methods used here for high throughput CC and accurate PH measurements are promising for continuously capturing crop growth in response to environmental changes.

This study demonstrates the application of a novel high-throughput phenotyping approach, i.e., imaging of CC and terrestrial laser scanning (TLS) of PH, to quickly identify winter wheat genotypic variations in the field. Preliminary results suggest new insights into modifying crop yield-related morphological traits and altering the duration of vegetative phase to gain yield increase in crop breeding.

Plenary session 2b:
Biotic stress resistance - COST session

Cause and effects; bottlenecks in the discovery and deployment of effectors and markers for the control of cereal Dothideomycete diseases

Richard Oliver

Centre for Crop Disease Management, Curtin University, Perth, Australia

Richard.oliver@curtin.edu.au

The most economically damaging foliar pathogens of wheat and barley in Australia are the Dothideomycetes *Parastagonospora nodorum* (Septoria nodorum blotch - SNB), *Pyrenophora tritici-repentis* (tan spot - TS) and *P. teres* (barley net blotch NB). Together with the related Dothideomycetes *Zymoseptoria tritici* (wheat Septoria tritici blotch) and *Ramularia collo-cygni* (barley Ramularia blotch) these pathogens cause substantial world-wide losses.

A decade ago, all these pathogens were, from a practical point of view, in the same state. No strong resistance was available and genetic analysis of resistance revealed multiple weak QTL that varied between season, localities and isolates. Few markers were in use. The discovery of a PtrToxA zenologue in *P. nodorum* ushered in a period of rapid improvement. A new dogma was established whereby these pathogens exerted pathogenicity by producing a number of effectors (Necrotrophic effectors) that induced a defence-like reaction in host germplasm carrying paradoxical sensitivity genes. The NEs were all small, secreted, cysteine-rich proteins. All that we needed to do was find all the effectors, and breed host germplasm that lacked all the sensitivity genes.

The dogma has had some notable successes. Breeding for TS and SNB in Australia has been substantially accelerated via the deployment of (for SNB) three effectors that, in retrospect, only partially fulfil the dogmatic criteria. SNB has turned out to be interestingly complex in its behaviour. Epistasis in effector expression has recently been substantiated and partially explained. Deployment of the effectors outside Australia has been much less successful. I will examine reasons for this observation.

The dogma has focussed research in other species. The search for small secreted, cys-rich proteins has been extensive but so far frustrating. Issues with genome assemblies, gene calling and effector properties have all been significant. More fundamentally, it has become apparent that the secretion into culture filtrates of stable proteins with NE activity may turn out to be exceptional rather than common. Strategies for the control of these intractable diseases needs to be driven by multifaceted studies that acknowledge the diverse properties of these pathogens and their effector repertoires.

Breeding pipeline for resistance to phyllody phytoplasmas in sesame

Bulent Uzun[a], Engin Yol[a], Rustem Ustun[a] and Cengiz Ikten[b]

[a] *Department of Field Crops, Faculty of Agriculture, Akdeniz University, Antalya, Turkey*
[b] *Department of Plant Protection, Faculty of Agriculture, Akdeniz University, Antalya, Turkey*

bulentuzun@akdeniz.edu.tr

Sesame (*Sesamum indicum* L.), is one of the most important oilseed crops because of its high oil content (50–60 %) and high ratio of unsaturated fatty acids. Sesame phyllody disease caused by phytoplasmas can be highly destructive in sesame growing areas of the world. This research was undertaken to select resistant genotypes to the disease. The disease agents were determined first by using nested PCR of 16S rRNA gene with the phytoplasma-specific universal primers P1/P7 and R16F2n/R2, respectively. Sequencing of the nested PCR products indicated that the 16SrII-D and 16SrIX-C group phytoplasmas were the agent of sesame phyllody (Catal et al. 2013, Ikten et al. 2014). For identification of insect vectors of the disease, above molecular analyses were also done for insect samples collected from sesame fields. *Orosius orientalis* was found to be the vector insect of the disease (Ikten et al. 2014). A diagnostic multiplex real-time qPCR assay was developed using TaqMan® chemistry based on detection of the 16S ribosomal RNA gene of phytoplasmas and the 18S ribosomal gene of sesame. The development of this qPCR assay provided a method for the rapid measurement of infection loads to identify resistance levels of sesame genotypes against phyllody phytoplasma disease. 542 sesame accessions originated from 29 different countries were tested against the disease using 1-5 scoring scale under field conditions in 2012 and the following year, 238 accessions were then again tested using the same scale. The disease epidemics in the field was encouraged by releasing vector insects fed on phyllody infected sesame plants at greenhouse. 30 accessions were selected from field studies as possible resistant sources to the disease according to 1-5 scoring scale. These accessions were further tested to confirm the resistance under greenhouse conditions against the disease. Each possible resistant accession was grown at greenhouse in cages with vector insects for establishing heavy disease pressure. DNA samples were taken at several developmental stages of plants in order to perform real-time qPCR analyses. Disease loads for each possible resistant accession other than visual scoring at greenhouse were identified by qPCR developed in this study. Two subsequent years of field studies with a large number of accessions, confirmative greenhouse experiments with disease bearing vector insects, and quantification of phyllody-causing phytoplasmas with qPCR in possible resistance accessions determined under field conditions, yielded two resistant sources namely ACS38 and ACS102. Plant registration for ACS38 is in progress.

Acknowledgements:
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Can the variation of secondary metabolite contents be part of carrot resistance to *Alternaria dauci?*

Koutouan C.[a], Yovanopoulos C.[c], Piquet M.[c], Hugueney P.[d], Halter D.[d], Claudel P.[d], Baltenweck R.[d], Hamama L.[a], Suel A.[b], Huet S.[b] , Peltier D.[a], Briard M.[b] and Le Clerc V.[b]

[a] *IRHS, SFR Quasav, University of Angers, France*
[b] *IRHS, SFR Quasav, Agrocampus Ouest, France*
[c] *IRHS, SFR Quasav, INRA, France*
[d] *UMR 1131, Métabolisme Secondaire de la Vigne, INRA-Université de Strasbourg, France*

claude.koutouan@angers.inra.fr

Combining different complementary mechanisms of resistance (i.e. different modes of action against the pathogen) through a QTL pyramiding strategy should be more efficient to develop durable resistant varieties than relying on a single mechanism. Based on various approaches we showed that different patterns of carrot resistance to *Alternaria dauci*, the fungus responsible for the most damaging leaf disease on this species, may exist (Boedo *et al*., 2010; Lecomte, 2013; Lecomte *et al*., 2014; Le Clerc *et al*., 2015). Some evidences support that some secondary metabolites could be involved in those different mechanisms of resistance. Indeed, laboratory experiments showed that jasmonic acid biosynthesis pathway could be involved in carrot resistance to *A. dauci* (Lecomte, 2013). Beside this, the inhibitory effect of falcarindiol on the development of *Alternaria dauci* was highlighted (Lecomte *et al*., 2012). We also detected differential accumulation of other secondary metabolites between resistant and susceptible cultivars in various growing conditions. To strengthen our assumptions that these secondary metabolites may be involved in carrot resistance to *A. dauci*, we analyzed the variation of these different types of secondary metabolites in segregating populations after *Alternaria dauci* infestation in field conditions. Some of them were identified as good candidates for metabolite QTL (mQTL) analyses and there colocalisation with previously detected resistance QTLs was investigated. Their potential implication in carrot resistance mechanisms to *A. dauci* will be discussed.

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Association mapping of durable resistance to Wheat powdery mildew

Javier Sánchez-Martín[a] and Beat Keller[a]

[a] *Department of Plant and Microbial Biology, University of Zürich, Switzerland*

javier.sanchezmartin@botinst.uzh.ch

Wheat is the most widely cultivated crop in the world ranking third regarding production quantity and playing an essential role in global food security. Under field conditions, wheat is subjected to abiotic and biotic stresses that reduce farm-yield below the genetically-determined production potential. Among biotic stresses, fungal diseases are major constraints for this crop. Wheat powdery mildew, caused by *Blumeria graminis* f.sp. *tritici (Bgt*), is one of the most devastating foliar diseases of wheat. Control of powdery mildew can be achieved through the use of major powdery mildew resistance (*Pm)* genes which confer race-specific resistance. This resistance, however, is frequently overcome by newly evolving pathogen races. Therefore, a constant search and transfer of new *Pm* genes is necessary to counter the continuous evolution of virulence of the pathogen. Alternatively, partial resistance, also called Adult Plant Resistance (APR), represents a more durable and broad-spectrum resistance conferred by quantitative resistance genes.

To identify potential new resistance sources, a collection of 506 geo-referenced inbred wheat accessions, genotyped by whole exome sequencing technology, is being evaluated both at seedling stage and at adult plant stage against powdery mildew. Association mapping (AM) analysis can reveal loci that contribute to race-specific resistance, potentially resulting in the identification of so far unknown *Pm* genes. In addition, AM should uncover accessions with adult stage, broad-spectrum resistance, a trait that is highly desired in breeding. Taken together, the output of this research will provide invaluable information for pre-breeding programs oriented towards breeding durable mildew resistance in wheat.

Down-regulation of Arabidopsis *DND1* orthologs in potato and tomato leads to broad-spectrum resistance to late blight and powdery mildew

Kaile Sun[a], Anne-Marie A. Wolters[a], Annelies E. H. M. Loonen[a], Robin P. Huibers[a], René van der Vlugt[b], Aska Goverse[c], Evert Jacobsen[a], Richard G. F. Visser[a] and Yuling Bai[a]

[a] *Wageningen UR Plant Breeding, Wageningen University and Research Centre, The Netherlands*
[b] *Laboratory of Virology, Wageningen University and Research Centre, The Netherlands*
[c] *Laboratory of Nematology, Wageningen University and Research Centre, The Netherlands*

kaile.sun@wur.nl

Multiple susceptibility genes (*S*), identified in *Arabidopsis*, have been shown to be functionally conserved in crop plants. Mutations in these *S* genes result in resistance to different pathogens, opening a new way to achieve plant disease resistance. The aim of this study was to investigate the role of *Defense No Death 1* (*DND1*) in susceptibility of tomato and potato to late blight (*Phytophthora infestans*). In *Arabidopsis*, the *dnd1* mutant has broad-spectrum resistance against several fungal, bacterial, and viral pathogens. However this mutation is also associated with a dwarfed phenotype. Using an RNAi approach, we silenced *AtDND1* orthologs in potato and tomato. Our results showed that silencing of the *DND1* ortholog in both crops resulted in resistance to the pathogenic oomycete *P. infestans* and to two powdery mildew species, *Oidium neolycopersici* and *Golovinomyces orontii*. The resistance to *P. infestans* in potato was effective to four different isolates although the level of resistance (complete or partial) was dependent on the aggressiveness of the isolate. In tomato, *DND1*-silenced plants showed a severe dwarf phenotype and autonecrosis, whereas *DND1*-silenced potato plants were not dwarfed and showed a less pronounced autonecrosis. Our results indicate that *S* gene function of *DND1* is conserved in tomato and potato. We discuss the possibilities of using RNAi silencing or loss-of function mutations of *DND1* orthologs, as well as additional *S* gene orthologs from *Arabidopsis*, to breed for resistance to pathogens in crop plants.

Plenary session 3:
Secondary metabolites

Optimizing plant secondary metabolite production

Johannes Novak[a]

[a] *Institute of Animal Nutrition and Functional Plant Compounds, University of Veterinary Medicine Vienna, Austria*

Johannes.Novak@vetmeduni.ac.at

Plant secondary metabolites (PSM) are by definition plant products with advantages in manifold functional areas of the plant (pollination, repellence of herbivores, etc.), but not necessary for the plants survival. Many of these compounds show effects (activities) outside their functional role in the plant, used by animals and humankind. Species most intensely exploited for PSM are summarized as medicinal and aromatic plants (MAPs). Of course, also optimizing for appearance (colour) and aroma of other crops like fruits and wine is an optimization of PSM and many resistance mechanisms are based on them. The enormous variety of PSM is still an important driving force for the development of new medicines. Of all anticancer medicines developed since the 1940s, 75% were based on natural molecules and 49% are still natural products or directly derived thereof (Newman and Cragg, 2012).

A major feature of MAP production is the enormous richness of plant species. In total, 52,885 plant species are used as MAPs by humankind, of which 5,000 to 6,000 species are of commercial interest (Schippmann et al., 2002). In Europe, 130 to 140 MAP species are cultivated on an area of around 200,000 ha (Pank, 1998).

As can be seen by those figures, most MAP species are still wild collected. However, in terms of quantity, the majority of PSM raw materials are originating from cultivation. There is a trend in transferring wild collected species into cultivation, especially in cases of rapidly increasing demand as seen in the past in yew (*Taxus baccata*) and St. John's wort (*Hypericum perforata*), difficult sourcing situations and too high collection pressures on natural resources.

Regarding breeding activities, we can distinguish two categories:

Breeding of species of high demand and higher cultivation areas like parsley, basil, and fennel, where (partially) advanced breeding strategies and technologies are used.

Domestication of wild collected species. Here, breeding is performed using simple selection strategies of wild collected accessions and is often not performed by professional breeders. However, breeding success can be big and often decides about the economic feasibility of cultivation.

A further characteristic of breeding plants for PSM is the determination of PSM and PSM-levels requiring intensive use of phytochemical analysis or – in some cases –organoleptic characterisations. Both approaches have severe limitations in high-throughput use, namely costs, working time and – in case of sensorial evaluations – operator fatigue. Therefore, fast and cheap analysis methods like fast chromatographic separation or spectroscopic methods (e.g. NIRS) are required for efficient breeding. In addition, molecular markers for PSM are in some cases possible but still limited due to limited molecular resources of MAPs.

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Stilbene biomarkers to breed resistant grape varieties against fungal diseases

Olivier Viret[a], Jean-Laurent Spring[a] and Katia Gindro[a]

[a] *Institute for Plant Production Sciences / Department for Plant protection field crops and vine, Viticulture and Oenology, Agroscope, Switzerland*

olivier.viret@agroscope.admin.ch

Since the introduction of powdery and downy mildews in Europe late 19th century, breeding resistant cultivars by hybridizing *V. vinifera* (susceptible) with other *Vitis* species (resistant), has been largely used. This led, in 1947, to >350'000 ha (23%) of the grapevine area in France cultivated with these hybrids. Because of the poor wine quality of this first generation of hybrids, legislation prohibited their cultivation for the production of quality wines until now. Recent investigations allowed sequencing the entire genome of grapevine, but no precise resistance genes are known yet for further introduction in susceptible *V. vinifera* cultivars. At the molecular level, the approach of marker-assisted-selection of QTL (Quantitative Trait Loci) for resistance to diseases is ongoing and could be correlated to resistant gene expression and further to define metabolites production in resistance mechanisms.

Infections of grapevine by fungal pathogens induce active defense reactions related to specific biochemical pathways, in addition to constitutive mechanisms belonging to the genome of the host plant. Stilbenic phytoalexins are described in different plant-fungal interactions leading to cell death, necrotic spots, generally reported as hypersensitive reactions. In grapevine, resveratrol was first identified as a key defense molecule but also as a strong antioxidant and as potential chemopreventive for human health, leading to the so-called French paradox. The biocide effect of resveratrol on the main grapevine pathogens is weak, but depending on the biochemical pathways, it is transformed either in highly toxic or harmless molecules. In the most cultivated *Vitis vinifera* cultivars, after fungal infection, resveratrol is transformed in piceide by glycosilation, having no effect on fungal particles. On the other hand, the same amount of resveratrol in resistant cultivars is transformed in pterostilbene, viniferins, hopeaphenol ampelopsins and E-vitisin B, highly toxic for the pathogens. Therefore, stilbenic phytoalexins are key defense molecules involved in the resistance of cultivars to the three major fungal pathogens, *Botrytis cinerea* (grey mold of grape), *Plasmopara viticola* (downy mildew) and *Erysiphe necator* (powdery mildew). These metabolites can be analyzed by HPLC to evaluate efficiently the ability of the grapevine plants to inhibit the development of fungal pathogens and their cytotoxic effects analyzed and evaluated by transmission electron microscopy. Resistant grapevine varieties, obtained by crossing new generation of hybrids with high quality *V. vinifera*, react very rapidly to infections by producing high concentrations of the most toxic stilbenes at the sites of infection.

In *V. vinifera* cultivars, the use of fungicides to control fungal diseases is unavoidable to produce high quality grape and the only true alternative to pesticides is the breeding of resistant new varieties. Since 1996, several back-crosses of new generation hybrids with *V. vinifera* have been performed and allowed the registration of Divico (Gamaret x Bronner) in Switzerland, highly resistant to downy mildew and grey mold, and moderately susceptible to powdery mildew, opening a new way to a more sustainable viticulture.

Breeding less allergenic spelt wheat with low FODMAP content

Ferenc Békés[a], Katalin Ács[b], Csaba Lantos[b] and János Pauk[b]

[a] *FBFD PTY LTD, Australia*
[b] *Cereal Research Non-profit Ltd./Department of Biotechnology, Hungary*

firinc47@gmail.com

Wheat sensitive individuals, who were able to consume products manufactured from spelt wheat (*Triticum spelta* L.) cultivated in Australia without any symptoms, became sick from identical products made from imported European spelt-. Detailed chemical composition of samples representing spelt varieties grown at different regions in Australia has been compared with those cultivated in Europe. While most of nutritive components of the two subpopulations did not show significant differences, two important difference have been found: the Australian cultivars contained significantly less soluble oligosaccharides (FODMAPs), and the soluble protein composition of one of the cultivar, selected and grown in Australia, called GWF spelt, showed marked quantitative and qualitative differences. Based on these preliminary results a larger survey has been carried out, monitoring the FODMAP levels of *T. spelta* and *T. aestivum* cultivars, grown at different locations in New South Wales, Australia, in 3 consecutive seasons. It was found that (1) *T. aestivum* samples showed always higher FODMAP levels than any spelt cultivars. (2) Variation of this lover levels of FODMAP content in the spelt samples was found to be significantly larger than the variation of FODMAP contents in *T. aestivum* samples. This observation was found to be the basis of screening large number of spelt varieties is using the FODMPAP content as parameter of selection. (3) GWF spelt was found to have the lowest FODMAP content among all samples in any growing site and harvest season. (4) The most remarkable difference in the soluble protein composition of GWF spelt compared to any *T. aestivum* or spelt wheat is that a mutation have been found in its gene coding the allergenic expensing protein (Breen et al, 2010). (5) It was found that the immune reactivity of IgE wheat positive sera from a normal Australian population is lower for spelt- compared to wheat regardless of their origin but much lower against GWF spelt containing the mutation in its expansion gene (Vu et al, 2014).

To increase the speed of generation changing in breeding and to improve the homogeneity of new selected spelt-line an effective in vitro androgenesis system was developed in spelt wheat. The anther culture protocol was described for a Hungarian genotype (GK Fehér), but the protocol was tested by other 4 spelt registered varieties, too. The number of anther culture-derived embryo-like structures (ELS) was 62ELS/100 anthers, from which we were able to regenerate green plantlets. The percentage of green plantlets production was 89.0% among the regenerated plantlets while the phenomenon of albinism was restricted (3.8/100 anthers). Altogether, over 500 *in vitro* green plantlets were produced from anther culture of different spelt genotypes. Based on ploidy level analyses from the haploid plantlets the doubled haploid plantlets were produced via colchicine treatment and getting seed after spontaneous chromosome doubling. The colchicine treated and spontaneous DH plants were grown up in greenhouse. The in vitro haploid induction system was integrated in the breeding programme of spelt wheat.

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Improving performance and tannin content of the forage legume sainfoin (*Onobrychis viciifolia*)

Roland Kölliker[a], Carsten Malisch[a], Katharina Kempf[a] and Andreas Lüscher[a]

[a] *Agroscope Institute for Sustainability Sciences, Zurich, Switzerland*

roland.koelliker@agroscope.admin.ch

Tannins are a highly diverse group of oligomeric polyphenolic compounds which accumulate in some plant species including grape vine, chicory or sainfoin. In ruminants, condensed tannins have been shown to have anthelmintic properties against gut parasites, to increase protein utilization, to prevent bloating and to even reduce methane emissions. Sainfoin, *Onobrychis viciifolia*, is a pluri-annual forage legume grown in pure stands or in mixture with grasses and other legumes. Its presumably high content of condensed tannins makes it a promising candidate for enrichment of ruminant diets with these valuable plant secondary compounds. Despite its advantages, a wide adoption of sainfoin is hampered by the often poor agronomic performance and the limited availability of sainfoin cultivars adapted to specific environmental conditions. In order to facilitate sainfoin cultivation in the future, we aimed at (i) optimising sainfoin cultivation, (ii) investigating tannin content and composition in different cultivars and across the season and (iii) developing knowledge and tools for sainfoin breeding.

In a replicated field experiment bi-species mixtures of sainfoin with six different partner species, each in three sowing densities (12.5, 25 or 50% of recommended sowing densities for pure stands) and two cutting frequencies, meadow fescue and perennial ryegrass allowed for high forage yields while at the same time maintaining high sainfoin share and low weed proportions in the mixture. Partner species sowing density had no influence on yield while increased cutting frequency led to better forage quality under constant yield. In a second field experiment, tannin concentration and composition varied considerably within and among the 27 sainfoin accessions tested. In general, condensed tannin concentration increased during summer and was higher in young leaves when compared to older leaves and stems.

By investigating progeny of artificial directed pair-wise reciprocal pollinations of selected plants using sequence related amplified polymorphism (SRAP) markers, self-fertilization rates of up to 65 % were observed. This was surprising for a presumably allogamous species and significantly higher than the selfing rates of 0 to 4 % observed under natural non-directed pollination. In order to provide tools for marker assisted selection, a set of 101 simple sequence repeat SSR markers was developed and marker – trait associations were analysed in segregating mapping populations.

In conclusion, we identified meadow fescue and perennial ryegrass as the best companion species for sainfoin cultivation. The insight gained on tannin concentration and composition together with the knowledge on self-fertilization and the molecular genetic tools generated will allow for optimised cultivation and breeding of sainfoin in the future.

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Using the *L. sativa* x *L. serriola* lettuce mapping population to direct breeding for flavour and nutrition

Carol Wagstaff[a], Richard Michelmore[b] and Martin J Chadwick[a]

[a] *Department of Food and Nutritional Sciences, University of Reading, UK*
[b] *The Genome Center, University of California, Davis, USA*

c.wagstaff@reading.ac.uk

The publication of crop genome sequences has paved the way for the development of a range of further genomic resources and investigation of many more commercially useful and fundamental traits. We have combined a traditional QTL mapping approach with an innovative computational pipeline to define genomic markers and putative regulatory genes for traits related to nutrition and flavour quality in lettuce. The study made use of the genetic diversity present in the *Lactuca sativa* cv. Salinas x *Lactuca serriola* mapping population; a fully annotated genome sequence exists for both parent lines and transcriptome sequence has been obtained from the progeny. We combined data from three experimental growing environments for a wide range of metabolite traits that were characterized using NMR and verified using complementary techniques. We subsequently used a trained human sensory panel to establish that our biochemical measurements were paralleled by the human olfactory system and to understand how the interaction of different metabolites associated with bitterness and sweetness were perceived by humans upon consumption. Our combined trait-to-gene and gene-to-trait approach has enabled the identification of putative regulatory genes for sesquiterpenoid lactones which are key metabolites in lettuce linked to antimicrobial, antiherbivory, human health benefits and bitterness perception. This refined approach has enabled us to differentiate between sesquiterpenoid lactones linked to either health benefits or taste, to the extent that pre-breeding markers have ben identified for use in molecular breeding programmes which aim to improve one trait without compromising the other.

Parallel oral presentations: Tuber and industrial crops

Screening of 14 potato genotypes for their adaptation to tropical medium altitude conditions

Awang Maharijaya[1,3], G.A. Wattimena[1], Muhammad Syukur[1], Sobir[1], Muhamad Khudori[2], Sulaeman Taufik[3] and Agus Purwito[1]

[1] Department of Agronomy and Horticulture, Bogor Agricultural University, Jalan Raya Darmaga Bogor, Indonesia
[3] Central Agro Mikro Potatoes, Garut, Indonesia
[4]Center for Tropical Horticulture Studies, Jalan Padjajaran Bogor, Indonesia

awang.maharijaya@gmail.com

Potato is one of the most important crops in the world, including some tropical countries. The limitation of land availability for potato production in highland areas of some tropical countries has steered the recent development of production technology of some important vegetables normally growing in highland area, including potatoes, to lower altitude areas. To increase the effectiveness of the production in lowland areas, varieties tolerant to those particular conditions are needed. Our study aimed at screening of potato genotypes for their adaptation to low-medium altitude conditions (300-700 above sea level).
We evaluated and compare the growth and production of 14 potato genotypes collected from several sources including potato wild relatives growing at 1500 versus 550 above sea level. We also evaluated the ability of genotypes to produce tubers in an *in vitro* setup. Our results showed that several genotypes of potato produced tubers at medium altitudes and high temperatures in *in vitro* laboratory conditions. We identified one genotype, derived by single seed descent from CIP394614 (*Solanum tuberosum*), which was superior for its ability to produce tubers under these conditions. Thus, it might be very suitable to be used in a breeding program to develop tolerant varieties of potato to low-medium highland.

Stability of resistance conferred by pyramiding two QRLs for *G. pallida* Pa2/3

Brian Rigney[1], Vivian Blok[2], Denis Griffin[1], **Dan Milbourne[1]**

[1]*Crop Science Department, Teagasc, Oak Park, Ireland,*
[2]*Cell and Molecular Sciences, The James Hutton Institute, Invergowrie, Dundee, DD2 5DA*

dan.milbourne@teagasc.ie

The potato cyst nematodes (PCN) *Globodera roscochiensis* and *Globodera pallida* are significant pests of potatoes worldwide. The most effective control methods are crop rotation and the deployment of resistant varieties. Complete resistance to *G. rostochiensis* based a single resistant gene, has successfully been integrated into many varieties. However resistance to *G. pallida* has not been as successful to date with current varieties only exhibiting partial resistance. Combining partially effective quantitative resistance loci (QRLs) can increase the strength and breadth of the resistance. We have previously demonstrated an additive effect on resistance on combining two QRLs from *Solanum tuberosum* spp *andigena (GpaIV$^{s}_{adg}$)* and *Solanum vernei (GpaV).* However populations of *G. pallida* can be quite divergent and it was unclear whether the relative effects of the individual QRLs and the combined additive effect would be consistent across different *G. pallida* Pa2/3 populations. Using a mapping population segregating for both QTLs, we examined the effect of the QRLs individually and combined on four UK-derived field populations of *G. pallida* pathotype Pa2/3, and found that the relative effects of the individual QRLs and the additive effect of the combination were consistent across all populations.

Diversity of starch related genes among potato cultivars

Friederike Trognitz [a], Samira Basyouni [a], Katharina Chvatal [a], Elisabeth Wischnewski [a] Marnik Wastyn [b] and Felix Fuchs [c]

[a] *Health & Environment Department/ AIT Austrian Institute of Technology, Austria*
[b] *AGRANA Research & Innovation Center, AGRANA, Austria*
[c] *NÖ Saatbaugenossenschaf, Austria*

friederike.trognitz@ait.ac.at

One of the main products from potato tubers is starch. Although the production of potato starch has declined in the last several years, potato starch is characterized by specific properties, which are essential in the food, paper adhesives, building and textile industries. Potato starch is of particularly value for its unique texture, neutral taste and high clarity compared to other starches. Potato starch granules usually consist of 20-25% amylose and 75-80% amylopectin and have a highly ordered structure (Vos-Scheperkeuter, et al. 1987). In the past decades attempts have been made to alter starch properties by changing levels of starch synthases (SS) and starch branching enzymes (SBE) through genetic modification (Schwall, et al. 2000). By mutation of the granule-bound starch synthase gene (*GBSS I*) amylose free potato have been obtained (Jacobsen, et al. 1989). The development of amylopectin free potato was less successful by modification of starch synthase II and III genes (*SS II* and *SS III*) but resulted in novel starch characteristics.

The aim of this project was to find natural allelic variation of starch related genes among different potato cultivars. Therefore, the sequences of the genes from the starch pathway were identified in the sequence of *S. tuberosum* group Phureja DM 1-3 (v3.4) (http://solanaceae.plantbiology.msu.edu/blast.shtml) and primers were designed to cover the whole genes.

About 300 different potato cultivars were collected and the genes *GBSS I*, *SBE*, *SS I* and the gene encoding the starch-granule-bound r1 protein (*SGBR1 I*) were amplified. The amplicons were pooled for each genotype and fragmented using NEXTERA XT library preparation kit (Illumina). Every library contained 96 samples and each library was sequenced on the Miseq sequencer (Illumina).

The fastq files were aligned against the reference genes using bowtie 2. After the alignment every exon site in the genes were analysed for changes in the amino acid sequence.

To obtain starch with different properties, changes in amino acids with different chemical properties or the introduction of stop codons were of particular interest. Each SNP was be verified by Sanger sequencing.

Potato genotypes containing low frequency alleles with differences in the amino acid sequences will be used for crossing experiments and in the progenies the desired SNP will be monitored to obtain the quadruplex status.

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Transcriptional dynamics during the sugar beet, *Beta vugaris* ssp. *vulgaris* - *Rhizoctonia solani* interaction

Louise Andersson[a], Thomas Kraft[a], Mark Varrelmann[b] and Christina Dixelius[c]

[a]*Syngenta Seeds AB, Sweden*
[b]*Institute for sugar beet research, IfZ, Germany*
[c]*Department of Plant Biology, Swedish University of Agricultural Sciences, SLU, Sweden*

louise.andersson@syngenta.com

Sugar beet, *Beta vulgaris* ssp. *vulgaris*, is an important crop of temperate climates and is grown for its high sugar content. Breeding in sugar beet is done to increase sugar content and root yield but also for improving disease resistance. One of the most important sugar beet pathogens is the soil-borne basidiomycete *Rhizoctonia solani*. This fungus is causing severe root rot in several sugar beet growing areas. The disease could partly be controlled with fungicides, but the timing of the application is difficult and the best way to control the disease is to grow resistant varieties. Partially resistant varieties are available and sold on several markets, especially in USA. The resistance in sugar beets to *R. solani* is quantitative, and no resistance genes are known. QTLs for *R. solani* resistance have been identified but there is a yield drag associated with the resistance trait. If the resistance genes underlying the known QTLs could be identified, then new molecular markers could be designed to improve the selection efficiency in the breeding work.
To better understand how the resistance gene mechanisms involved in this plant-pathogen interaction works, a transcriptome analysis study was performed. Four sugar beet genotypes with different resistant/susceptibility levels were inoculated with *R. solani* in a greenhouse bioassay. Root samples were harvested prior to inoculation and at two and five days after inoculation. RNA was extracted and pair-end 100bp libraries were sequenced on an Illumina HI-seq 2500 platform. Transcriptreads were mapped to the sugar beet reference genome. Heatmaps were generated using the statistical software package R v3.2.3.
Differentially expressed genes in resistant compared to susceptible genotypes at the different time-points after inoculation were studied. The results indicate an active transcriptional response early on to the pathogen regardless of genetic background. In a later response there are classes of genes uniquely regulated in either the resistant or susceptible lines. A core set of genes more highly expressed in resistant lines includes genes with sequence similarity to protein domains of disease resistance genes. For example cytochrome P450, genes with oxidoreductase activity, defense related genes, several protein kinases, several genes with AP2/EREBP domains common to PR genes, WRKY transcription factors and glutathione S-transferases.

Genetic diversity in a large Miscanthus germplasm collection

Susanne Barth[a], Manfred Klaas[a], Paul Cormican[b], Cora Muennich[c], and Trevor R Hodkinson[d]

[a] *Teagasc Crops Environment and Land Use Programme, Oak Park Research Centre Carlow, Ireland*
[b] *Teagasc Animal and Bioscience Research, Department, Animal & Grassland Research and Innovation Centre, Grange, Dunsany, Co. Meath, Ireland*
c Biotechnik und Pflanzenvermehrung GmbH, Wanzleben-Börde, Germany
[d] *Botany, School of Natural Sciences, Trinity College Dublin, University of Dublin, Ireland*

susanne.barth@teagasc.ie

Miscanthus spp. has arrived in Europe a century ago as a plant with ornamental interest and was distributed in Botanic Gardens. *Miscanthus* has a wide range of applications from raw material for the paper industry, thatching, animal feed and also as bioenergy crop species. *Miscanthus* is thought to have originated in South East Asia and has a range of species. Currently the commercially most successful species is *Miscanthus x giganteus*. Unfortunately only 1 clone of this species has initially found wide distribution as bioenergy crop which makes it vulnerable to potential diseases and pests. Widening the genetic basis of *Miscanthus* by either using the other main species like *M. sinensis, M. sacchariflorus* or the production of novel *M x giganteus* germplasm by hybridizing *M. sinensis* with *M. sacchariflorus* are therefore desirable to provide a wider genetic basis with potentially higher resilience to a number of abiotic and biotic stresses.
We report on the genetic diversity of novel collections of Miscanthus in Siberia and in China and their genetic relationships to collections which have been in Europe for the last 40 years. These novel collections have been made in 2014 as part of the EU FP7 funded GrassMargins project. We have used the genotyping by sequencing technique to generate genetic diversity data and also have analysed the ploidy of our collection by flow cytometry. We have identified some natural hybrids in Russia.

Genetic diversity of *Patellifolia patellaris* from Southeast Spain, a crop wild relative of cultivated beets

Lothar Frese[a], Lorenz Bülow[a], Marion Nachtigall[a], Maria Luisa Rubio Teso[b], Maria Cristina Duarte[c], Elena Rey[a], and Maria José Iriondo[b]

[a]*Institute for Breeding Research on Agricultural Crops, Julius Kühn-Institut (JKI), Germany*
[b]*Área de Biodiversidad y Conservación, Universidad Rey Juan Carlos (URJC), Spain*
[c]*Centre for Ecology, Evolution and Environmental Changes (CE3C), University of Lisbon, Portugal*

lothar.frese@jki.bund.de

The genus *Patellifolia* (former *Beta* section *Procumbentes*) consists of three species *P. patellaris*, *P. procumbens* and *P. webbiana*. The latter two can only be found on some of the islands of the Macaronesian archipelagos (Madeira, Canary Islands), while *P. patellaris* also occurs on the Cape Verdean Islands and along the coastline of the Maghreb region and the Iberian Peninsula. The genus has high potential as a source of resistances to a range of disease agents. The gene Hs^{pro-1} was introgressed from *P. procumbens* and *P. webbiana* into the sugar beet in the 1980s to control the sugar beet cyst nematode *Heterodera schachtii* (Löptien, 1984, Lange *et al*., 1993). Pathotypes able to break the resistance gene are known since 1998 (Müller, 1998). The durable control of the cyst nematode is based on two approaches: (i) the sustainable deployment of Hs^{pro-1} in crop production (Brun *et al.* 2011) and (ii) the search for novel genetic variation in the crop wild relatives. Detailed knowledge of the structures of genetic diversity can facilitate the detection of novel gene variants. So far, genetic diversity of only a few occurrences of *P. patellaris* has been investigated (El Bahloul and Gaboun, 2013). Therefore a comprehensive study was started in 2015 to characterize genetic diversity in a larger set of *Patellifolia* occurrences.

Here, we report on the development of new SSR and on the investigation of 10 occurrences of *P. patellaris* sampled on the Iberian Peninsula. The genomic sequence from *P. procumbens* was screened for SSRs and 3648 SSRs were identified. A subset of 53 was validated of which 24 proved to be polymorphic in reference samples of *P. patellaris*, *P. procumbens* as well as *P. webbiana*. The number of alleles in the reference samples ranged from 57 in *P. patellaris*, 187 for *P. procumbens* to 202 in *P. webbiana* (Nachtigall *et al*., submitted). These 24 new markers were used to study genetic diversity of the 10 occurrences. Within this set of 272 individual plants, a total of 242 alleles was detected. The number of alleles per marker ranged between two (JKIPat08) and 16 (JKIPat11) and was highest in occurrences sampled in the region of Almeria. A factorial analysis and cluster analysis was performed to investigate the variation patterns. A clear genetic distinctiveness between occurrences was observed. So far, our study revealed a pattern of spatial differentiation between occurrences that could be explained by an independent colonisation of the sites.

The study has partly been funded by the ECPGR (http://www.ecpgr.cgiar.org/working-groups/beta/gedipa/)

Parallel oral presentations:
Cereals

The quest for durable resistance to rust diseases in barley

Peter M Dracatos[a], Davinder Singh[a] and Robert F Park[a]

[a] *Plant Breeding Institute, Faculty of Agriculture & Environment, University of Sydney, Australia*

peter.dracatos@sydney.edu.au

In Australia, barley is the second most important cereal crop, with an estimated annual production of seven million tonnes. With a reputation for consistent, contaminant free high yielding barley varieties, Australia currently has the highest malting selection rate of all barley exporting countries. However, barley is susceptible to numerous foliar diseases including the *Puccinia* rust pathogens that dramatically reduce yield during severe epidemics. Leaf rust, caused by *Puccinia hordei*, is one of the most destructive foliar diseases of barley, with consistent annual epidemics in all growing regions that have resulted in significant yield losses in many barley growing regions. The barley stripe rust pathogen (*P. striiformis* f. sp. *hordei*) is currently a significant exotic threat to the Australian barley industry. Field testing at CIMMYT (Toluca, Mexico) over the past 10 years has shown that more than 70% of Australian barley varieties are vulnerable to this disease.

Highly protective, single major or all-stage resistance genes have had limited success in agriculture due to the rapid emergence of pathogen races with matching virulence. For leaf rust, 23 resistance genes have been formally catalogued, including 21 all-stage genes, most of which have been overcome by *P. hordei*. Resistance expressed at later developmental growth stages, known as adult plant resistance (APR), in contrast, has mainly been reported to be race non-specific and in wheat broadly effective to multiple rust pathogens (pleiotropic). Unlike wheat where numerous APR genes have been catalogued and in three (*Lr34, Lr67, Yr36*) cases cloned, understanding of APR to leaf rust in barley is poor. While many APR sources have been identified, only two have been catalogued in barley viz. *Rph20* and *Rph23*. We phenotyped five doubled haploid mapping populations in multiple field environments over three years. All parents were seedling susceptible to the field *P. hordei* pathotype 5457P+, and in all populations, one parent carried *Rph20*. All five mapping populations, in addition to a diverse international barley association mapping panel, were genotyped using approximately 20,000 DArT-Seq markers. Both marker-trait associations and QTL analyses identified at least four new consistently detected APR loci that were additive in combination with *Rph20*. The development of PCR-based markers for each new locus enabled the analysis and identification of each known and unknown APR within both Australian and International germplasm. This paper reports the identification, characterisation and gene isolation of multiple APR sources in barley to leaf rust and the use of marker assisted selection to identify new APR sources in international germplasm. The current approach for pre-emptive breeding in Australia to barley stripe rust will also be discussed. In conclusion, the deployment of multiple additive adult and all-stage resistance sources in combination is the most desirable solution for durable resistance to rust diseases in barley. Our search for epistatic loci that confer resistance against multiple pathogens continues.

Identifying the best variety at each site with climatic-limitation covariates

Juan M. Herrera[a], Annelie Holzkämper[a], Lilia Levy-Häner[a] and Didier Pellet[a]

[a]*Institute for Plant Production Sciences, Agroscope, Switzerland*

juan.herrera@agroscope.admin.ch

Proper characterization of environments is fundamental for understanding genotype by environmental interactions (G × E) and predict accurately genotypic performance at large spatial scales. Here we evaluated if derived climatic-limitations from gridded weather data can be used as covariates to predict genotypic performance at a country scale.
We derived environmental limiting factors from daily weather data for critical wheat growth phases using a climatic suitability model (Holzkämper et al. 2015). The limiting factors that account for the effects of environmental variables on wheat productivity and phenology were then integrated into a matrix of environmental factors that related environments with grain yield observations. Prediction accuracy was evaluated through correlations between predicted and observed yield for six winter wheat genotypes grown at 10 sites (stations) during three years. Accuracies obtained for the different genotypes ranged from r=0.66 to r=0.92, which is well within the range of values reported for other studies of wheat. This demonstrated that environmental limiting factors obtained from gridded weather data allow to predict genotypic performance in large areas and represent them in maps. Specific genotypic performance in areas of 2x2 km was visualized in maps to identify the most suitable genotype for each area of Switzerland where wheat cropping is feasible.

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Prioritizing QTLs for heat stress tolerance using cell membrane stability in durum wheat

Ghasemali Nazemi[a], Silvio Salvi[b] and Roberto Tuberosa[b]

[a] *Department of Agriculture, Haji abad Branch, Islamic Azad University, Haji abad, Iran.*
[b] *Department of Agricultural Sciences (DipSA), University of Bologna, Bologna, Italy.*

nazemigh@iauha.ac.ir

Heat stress due to increased temperature is an agricultural problem in many areas of world. In this study, the association mapping (AM) strategy based on a panel of 183 elite of durum wheat accessions was deployed in order to dissect the genetic control and identify QTLs for heat stress tolerance. Cell membrane stability (CMS) was recorded as a proxy index to evaluate the response to heat stress in a three-step experiment: constitutive heat stress response, acquired heat stress response and constitutive-acquired heat stress response. Significant differences among genotypes were observed for all measured CMS traits. The panel was profiled with simple sequence repeat, Diversity Arrays Technology and sequence-tagged site markers (957 markers in total). Thirty four single marker/QTL regions were located on all chromosomes; four major QTLs (LOD ≥ 3) for constitutive heat stress response were detected on chromosomes 5A, 6A, 7B, while one QTL for constitutive-acquired heat stress response was detected on chromosome 6B. It is interesting that a higher number (nearly double) of QTLs were detected for constitutive heat response trait (heat shock applied to detached leaves) as compared to the two traits involving acquired responses, measured on living plants pre-adapted to high temperatures. The reason for this result could be that the constitutive response on detached leaves has a less complex genetic basis and higher heritability than acquired resistance observed in intact plants. The wide range of genetic variation and the limited influence of population structure support the reliability of our results and prompt for additional finer investigations of the physiological bases underlying these QTLs, towards their exploitation in breeding.

WheatOfChange - challenging wheat yield stagnation

Borislav Kobiljski, Zorica Jestrović, Ljiljana Brbaklić, and Gojko Mladenović

Biogranum, Serbia

borislav.kobiljski@biogranum.com

The current work aims to present a concept/solution called "WheatOfChange", which was developed through a Horizon 2020 project in order to overcome the problem of stagnating wheat yield that has been challenging stable production over the last 20 years.

Our main objective is to produce promising crosses that can be successfully implemented into breeding programs worldwide. The concept is based on the idea of selecting the most promising parents using extensive knowledge obtained from screening over 10,000 new and old varieties, advanced lines and genotypes from Southeast Europe, Balkans, Russia, Ukraine, etc. The studied samples come from 15 different growing seasons and from various environments.

When these carefully selected parents are crossed with newly developed varieties released by European wheat breeding companies, initial breeding material with exceptional genetic background is obtained. This has a significant potential to challenge the excessively narrowing genetic variability that is one of the main factors responsible for the present wheat yield stagnation.

The last 40 years' yields produced by Serbian wheat varieties in more than 35 countries (EU-28, Russia, Ukraine, Kazakhstan, Uzbekistan, Turkey, Iran, Canada, Argentina, etc.), which were developed by using a similar concept, confirm that merging desirable genetic variability from East and West can produce excellent initial wheat genetic material for further selection in different environments around the world.

Our proposed solution is a platform named "WheatOfChange" that rests on three main pillars: "BigWheat", which identifies crosses with more than 10% (up to 80%) increase in grain size and weight compared to better parent; "Grain4Gain" offers an opportunity to wheat breeding programs worldwide to enhance winter cereals yield by introducing novel genetic variability to be used in further selection for desired environments; and "CCB" service, which offers an opportunity for cereal crossbreeding by order.

Currently, F1 seeds from 1,549 crosses are available including pedigree, plant height, heading time, heterosis percent (if present), and early vigour data. We are planning to produce between 2,500 and 5,000 new crosses per year, and to have 20,000 crosses available on our horizon by 2020.

Association genetics and validation strategies in European wheat varieties

Marion S. Röder[a], Jie Ling[a], Christine Zanke[a], Bernd Rodemann[b], Christiane Balko[c], Frank Ordon[d], Sonja Kollers[e], Viktor Korzun[e], Erhard Ebmeyer[e], Odile Argillier[f], Gunther Stiewe[g], Thomas Zschäckel[g], Jörg Plieske[h] and Martin W. Ganal[h]

[a] *Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany*
[b] *Julius Kühn Institute (JKI), Braunschweig, Germany;* [c] *JKI, Groß Lüsewitz, Germany*
[d] *JKI, Quedlinburg, Germany;* [e] *KWS LOCHOW GMBH, Bergen, Germany*
[f] *Syngenta France S.A.S., Orgerus, France;* [g] *Syngenta Seeds GmbH, Bad Salzuflen, Germany*
[h] *TraitGenetics GmbH, Gatersleben, Germany (Project coordinator)*

roder@ipk-gatersleben.de

An overview about two long term studies (WHEAT, 2008-2011 and VALID, 2011-2015) regarding genome-wide association mapping in European winter wheat will be presented. A comprehensive dataset based on multi-environmental field data was developed for yield, other agronomic traits including resistance to biotic and abiotic stresses and baking quality. The phenotypic data were generated in replicated field trials at different locations and over several years respectively, using a panel of 358 European winter wheat and 14 spring wheat varieties mainly released between 2000 and 2005.
The genotypic data consisted of a set of microsatellite markers and two sets of mapped Illumina iSelect 90K Infinium and 35K Affymetrix SNP markers that reflect the genome-wide haplotype diversity. Furthermore, specific markers for major phenology traits (such as *Rht*, *Ppd* and others) were included. The analysis of marker-trait associations was carried out by using a mixed linear model and a kinship matrix based on microsatellite markers for population stratification correction.
For validation of identified marker-trait associations (MTAs), a second wheat panel consisting of 133 more recent (mainly released in 2005 to 2010) winter wheat varieties was established and tested in two years of field trials. DH-populations and BC2S2-lines developed via marker-assisted selection, have been used for the validation of selected QTL-loci for yield and resistance to *Fusarium* and *Septoria*.

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Efficiency of molecular marker tags in improving salt tolerance in rice using Forward Breeding MAS approach

Thirumeni Saminadane [a], Geddam Satyadevi [a], Paramasivam Krishnaswamy [a], Karthick Jothyganapathy [a], John D. Platten [b], Rakesh K. Singh [b] and Umashankar Singh [b]

[a] *Department of Plant Breeding and Genetics, Pandit Jawaharlal Nehru College of Agriculture and Research Institute, Karaikal, India.*
[b]*International Rice Research Institute, Los Banos, Philippines*

s_thirumeni@rediffmail.com

Breeding for salt tolerance, a major abiotic stress limiting productivity of many crops including rice, is a best option to increase rice production in such areas. As it is complex physiologically and genetically its improvement is slow, but molecular markers allow these genetics to be mapped and effects of loci identified. Platten et al., (2013) reported 11 major QTL (including *Saltol)* for various traits related to seedling stage salt tolerance in FL 478. Forward breeding MAS is considered better to Marker Assisted Backcross (MABC) because superior gene combinations, apart from introgression of targeted gene/QTL, are created without resorting to tedious background selection and high volume crossing during backcrosses. The present study was, therefore, attempted to test the efficacy of molecular markers linked to these QTL through phenotypic and marker assisted selection in ADT 45/FL 478 cross and fix salt tolerant white pericarp rice in the early generations. ADT45, a high yielding but salt-sensitive cultivar with white pericarp, was used as a female parent while FL 478 (a RIL of IR 29/ Pokkali), a salt tolerant donor, was used as a male parent to generate F_1 and subsequently a segregating population. Segregating progenies of the F_2 to F_5 generations were phenotyped first for seedling stage tolerance in hydroponics culture at EC 12 dSm^{-1} in the glass house adopting the procedure of Gregorio et al., (1997). Data on salinity (SES) score and physiological traits: shoot Na, K contents and their ratio and chlorophyll content were recorded. Subsequently, tolerant plants, identified in the glass house, were planted in the field in normal condition to evaluate agronomic traits. These plants were genotyped in the $F_3 – F_5$ using markers linked to six QTLs: two on Ch. 1 and four on Ch. 3 for salt tolerance and one functional marker (on Ch. 7) for pericarp colour. Genotyping was done in the $F_3 – F_5$, instead of F_2 to recover more of desirable homozygotes. A total of 185 plants that scored 1 & 3, on the SES scale, were selected from 1212 F_2 individuals, evaluated for seedling stage tolerance in hydroponics culture at EC 12 dSm^{-1} and from these 101 tolerant plants were identified as superior based on agronomic traits including grain yield. These selected 101 families were further screened in the F_3 as in F_2 and 280 plants, with score 1 or 3 (on single plant basis) were selected. This procedure was repeated in F_4 and F_5. Out of 280 plants, 260 were positive for *Saltol* QTL and the remaining 20 were positive for other QTLs. All tolerant plants possessed a minimum of two QTLs. In the F_5, 52 homozygous salt tolerant lines for these QTLs in various combinations of 2 to 6 QTLs were isolated. The level of tolerance in lines with differing numbers of QTLs correlated significantly with contents of chlorophyll and Na/ K ratio. 22 of these were white pericarp plants and nine promising lines are advanced for further testing. Therefore, it is inferred that molecular tags linked to 6 QTL and one functional marker for pericarp colour were very effective in recovering all the salt tolerant lines with white pericarp, as evidenced from identification of promising salt tolerant homozygous lines early in the generation.

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Breeding towards improving an African indigenous crop: the case of Tef

Zerihun Tadele[a,b], Gina Cannarozzi[a], Solomon Chanyalew[c] and Kebebew Assefa[c]

[a] *Institute of Plant Sciences, University of Bern, Switzerland*
[b] *Institute of Biotechnology, Addis Ababa University, Ethiopia*
[c] *Debre Zeit Agricultural Research Centre, Ethiopian Institute of Agricultural Research, Ethiopia*

zerihun.tadele@ips.unibe.ch

African indigenous crops are considered to be climate smart crops due to their high tolerance to several environmental stresses, especially to drought. Among these crops, tef (*Eragrostis tef*) is one of the most important cereal crops cultivated in the Horn of Africa, particularly in Ethiopia where it is annually grown on three million hectares of land and is a staple food for about 50 million people. Despite its importance, tef has a lower yield than most other cereals, mainly due to the widespread use of unimproved cultivars. The Tef Improvement Project at the University of Bern, implements diverse genetic and genomic tools to develop cultivars with desirable agronomic and nutritional traits. The first semi-dwarf and lodging-tolerant tef cultivars developed by the project using mutation breeding and TILLING have been tested at several locations in Ethiopia and soon will be released to the farming community. Two promising lines with high drought tolerance have been introgressed to high-yielding improved cultivars and the advanced F_6 populations are being investigated at several drought-prone areas in Ethiopia. In collaboration with key partners, the draft sequence of the tef genome has been completed and is currently being utilized to identify and isolate genes responsible for useful agronomic and nutritional traits. Differentially regulated genes under contrasting moisture regimes have been identified using RNAseq. The Project is also involved in human capacity building through organizing short- and long-term training for farmers, developmental workers and researchers in Ethiopia. In general, the Project has established a strong partnership with the National Agricultural Research System in Ethiopia, not only to develop improved cultivars but also to promote their dissemination to farmers across the country.

Parallel oral presentations:
Fodder crops

Advanced genotyping in three successive generations of the allotetraploid *Festuca pratensis* × *Lolium perenne* hybrid

Joanna Chojnicka[a], Katarzyna Molik[b], Hanna Ćwiek-Kupczyńska[a], Paweł Krajewski [a], Katarzyna Bzdęga[c], Agnieszka Janiak[c], Zbigniew Zwierzykowski[a] and Tomasz Książczyk[a]

[a]*Institute of Plant Genetics of the Polish Academy of Sciences, Poznan, Poland*
[b] *West-Pomeranian University of Technology, Szczecin, Poland*
[c] *University of Silesia in Katowice, Poland*

jcho@igr.poznan.pl

The species within the *Festuca-Lolium* complex are the most important forage grasses of the temperate climate region. They constitute a model research subject due to, among other reasons, their high ability to create intergeneric hybrids between the two genera and to initiate evolutionary processes within the limits of the introgression or amphiploid recombination. Genome differences between *Festuca* and *Lolium* species as well as their intergeneric hybrids can be detected either by cytogenetic or molecular studies. Physical mapping of genes responsible for quality traits requires well established cytogenetic maps and chromosome identification in the *Festuca* × *Lolium* hybrids. Localization of different DNA sequences using fluorescence *in situ* hybridization (FISH) together with an assignment of known chromosomal markers to corresponding genomes by genomic *in situ* hybridization (GISH) allows determination of markers for particular chromosomes. This is especially important for *Festuca* and *Lolium* species, which have chromosomes with a reduced number of chromosome-specific physical markers. In our ongoing research we have focused on the genomic structure and the identification of complete and recombined ribosomal DNA (rDNA)-bearing chromosomes, and the dynamics of chromosomal number and position of rDNA loci in the F_1-F_3 generations derived from selected individuals of F_1 and F_2 hybrids of *Festuca pratensis* ($2n=4x=28$) × *Lolium perenne* ($2n=4x=28$). The results have revealed that plants of the three initial generations share various rDNA loci profiles with chromosome structural changes, possibly as a result of chromosomal inter- and intra- rearrangements. For the first time herein, all the plants from the initial generations of *F. pratensis* × *L. perenne* hybrids studied cytogenetically have also been analysed in respect of PCR-based inter-simple sequence repeat (ISSR) markers and statistical differences to find relations between genome structure and genetic variation. We have also compared distributions of cytological markers between F_2 and F_3 (sub)combinations and distributions of selected cytological markers for all F_2 and F_3 plants, showing statistically significant differences between *L. perenne* and *F. pratensis* genomes concerning the number of rDNA-bearing chromosomes among F_2 (sub)combinations. Still, little is known about parental chromosome identification in the *Festuca-Lolium* complex or any precise monitoring of recognized and unrecognized rearranged chromosomes of both parental genomes. Therefore, FISH with other DNA sequences as probes (e.g. genome species-specific DNA sequences) is being carried out for physical mapping and working out new chromosome/genome-based markers for *Festuca*- and *Lolium*-genome-like chromosomes in *Festuca* × *Lolium* hybrids. The study of (cyto)genetic variation in genome architecture of *Festulolium* cultivars can help to understand the extensive recombination between chromosomes of the parental genomes underlying a novel genome-dependent range and type of chromosome variation in plants of the F_1-F_3 generations derived from *F. pratensis* × *L. perenne* hybrid.

Development of TILLING in outcrossing forage crops

Chloe Manzanares[a], Michael Ruckle[a], Steven Yates[a], Michael Meier[a] and Bruno Studer[a]

[a] *Institute for Agricultural Sciences, ETH-Zurich, Switzerland*

chloe.manzanares@usys.ethz.ch

Mutation breeding has a long-standing history as a powerful tool to generate genetic diversity for crop breeding programs. Up until the turn of the century, mutation breeding has relied on phenotypic selection, thus limiting its benefits to dominant traits in outcrossing species. In the post-genomic era, methods for TILLING (Targeting Induced Local Lesions IN Genomes) have been established in model plant species and have more recently been adapted to second generation sequencing technologies to maximize the potential of mutation detection and its utility to major crop species. However, the identification of induced mutations by multi-dimensional DNA pooling remains challenging in genetically diverse populations of outcrossing forage crops (Manzanares et al, 2016).
The recent publication of the perennial ryegrass (*Lolium perenne* L.) and the red clover (*Trifolium pretense* L.) genome sequences, coupled with advances in single molecule sequencing (SMS) has expanded the utility of modern TILLING to forage crops. Here we describe the development of TILLING populations for perennial ryegrass and red clover as well as novel methods to overcome challenges such as mutagenesis, assessment of the mutation frequency, chimerism, and genetic diversity in the starting population. In addition, we describe a sequencing strategy for mutation detection based on SMS. These TILLING strategies provide a novel reverse genetic resource which will be used to study important agronomical genes involved in biological mechanisms such as self-incompatibility, carbohydrate synthesis, abiotic stress… The use of TILLING to identify new alleles and gene functions will directly benefit forage research and breeding communities.

Reference:
Manzanares, C., Yates, S.A., Ruckle, M., Nay, M., Studer, B., 2016. TILLING in forage grasses for gene discovery and breeding improvement. New Biotechnology, in press

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A breeder's perspective of exploiting the potential that genomics have to offer

Sarah A. Palmer, Leif Skøt and Alan Lovatt

[a] *IBERS, Aberystwyth University, UK*

sap42@aber.ac.uk

Genomic selection (GS) offers considerable power as a selection tool in plant breeding, by estimating the genomic potential of an individual to perform before any trait measurement is made. That said, the additional investment to apply GS in routine breeding must be recouped by a measureable increase in breeding gain per generation for economically important traits. Strategies for maximising the effect of GS on breeding gain will differ between breeding systems, but the following generalisations can be made. GS can increase breeding gain by: a) identifying elite progeny faster, b) increasing the manageable base population number, c) increasing selection pressure, d) reducing the generation time or e) any combination of the above. All strategies depend on the accuracy of the genomic estimated breeding value (GEBV) which will only ever be as accurate as the training set from which they were derived, which in turn is affected by the genetic potential for a given trait and measurement of that trait, or heritability. Recurrent selection breeding systems are particularly suited to GS as the cycling nature of interbreeding and reselection generation after generation, builds GEBV accuracy over generations in parallel with the breeding program. This presentation will reflect on the development of GS for genotypic recurrent selection populations of diploid perennial ryegrasses; the relative success considering low and high heritability traits; strategies for implementation in commercial breeding using a cost/benefit analysis; and further enhancements (including new trait introduction) using accelerated recurrent cycling and novel phenomics.

Development of high energy red clover

Michael E. Ruckle[a], Michael Meier[a], Lea Frey[a], Samuel C. Zeeman[a] and Bruno Studer[a]

[a] *Institute for Agricultural Sciences, ETH-Zurich, Switzerland*

mruckle@ethz.ch

Red clover (*Trifolium pratense* L.) is one of the most important forage legumes worldwide and like other forage legumes, it offers a highly valuable feed source for ruminant livestock. Although red clover has a relatively high biomass potential, it lacks the fermentable high-energy carbohydrates required to meet the productivity potential of modern livestock breeds. Therefore, red clover-based diets are supplemented with high-energy maize and cereals that are often derived from unsustainable foreign supply chains. Red clover accumulates starch in its leaves during the day as a temporary carbon store of photosynthesis and remobilizes it to support metabolism and growth at night. Although plant leaves were first reported to accumulate starch over a century ago, leaf starch content has yet to be exploited as an agronomic trait in forage crops.

To improve the overall leaf starch content, we tested a genetically diverse population of red clover and found that both daytime starch accumulation and nighttime starch degradation have a high degree of natural variation. Moreover, red clover has the genetic potential to accumulate up to one third of its dry mass as starch. Interestingly, we did not observe a correlation between starch content and biomass. As a diurnal trait, variation in leaf starch content is strongly influenced by photoperiod and light intensity. To overcome the strong gene by environment interaction of this trait, we identified genotypes with greater diurnal stability. Additionally, we applied the current understanding of leaf carbohydrate metabolism from the model plant Arabidopsis and the recently published red clover genome to direct an advanced breeding approach based on Targeting Induced Local Lesions in Genomes (TILLING). With TILLING, we aim to identify beneficial alleles in genes required for diurnal starch mobilization, which are expected to further sequester starch in the leaf. These novel alleles obtained through TILLING will be combined with germplasm selected for diurnal stability to develop a high-energy forage trait. Such a trait is expected to be readily integrated into advanced breeding material, and will provide a feed source to significantly improve the economic and environmental sustainability of ruminant livestock production.

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Genetic analysis of resistance to Crown rust in a genotype of perennial ryegrass

Franz Xaver Schubiger and Beat Boller

Institute for Sustainability Sciences, Agroscope, Switzerland

franz.schubiger@agroscope.admin.ch

Perennial ryegrass (*Lolium perenne* L.) is a major component of temperate grassland. It is a highly outcrossing, wind-pollinated species exhibiting a gametophytic system of self-incompatibility (Cornish et al. 1979). Crown rust, caused by *Puccinia coronata* f. sp. *lolii* is a common disease of perennial ryegrass in Europe.

To study the inheritance of resistance to crown rust in perennial ryegrass, we crossed a resistant, diploid genotype of our breeding material (A6128/05) with a susceptible genotype of the cultivar Aurora to generate a F_1 population. Out of this F_1 population, we selected two genotypes (I and II) based on the reaction to single-pustule isolates (SPI) of crown rust. Both genotypes were backcrossed with a second, susceptible Aurora genotype (to avoid inbreeding a different susceptible genotype was used) to generate two F_2 populations. A resistant genotype out of the F_2 population derived from the genotype I was used to generate a F3, F4 and F5 population by backcrossing with a susceptible Aurora genotype.

The parents and F_1 to F_5 populations were screened for their response to three to five SPI of crown rust in a detached-leaf segment test under growth chamber conditions (Schubiger and Boller 2016).

The resistance donor A6128/05 was resistant to all of the five SPI used.

In the F_5 population, derived from a cross with the genotype I, a good fit to a segregation ratio of 1R:1S (resistant : susceptible) was observed for the three SPI 12_08, 523_04 and 531_01. Out of 112 genotypes tested, 49 % were resistant. The same genotypes were resistant, regardless of the SPI used. SPI 529_01 and 532_02 were virulent to all of the progeny.

The frequency of resistant and susceptible plants within the F_2 population, derived from the genotype II, resulted in a ratio of 1R:1S for SPI 12_08, 529_01 and 532_02. Out of 112 genotypes tested, 53 % were resistant. Nearly all the resistant genotypes were identical, irrespective of the SPI used. Only one genotype differed in the response. In contrast, the F_2 progeny was susceptible to SPI 523_04 and 531_01.

These results provide evidence that at least two different dominant genes for crown rust resistance occur in the resistance donor A6128/05. The two R-genes are different in their reaction to the SPI tested. The first gene confers resistance to SPI 12_08, 523_04 and 531_01, but not to 529_01 and 532_02. The second gene proved to be effective against SPI 12_08, 529_01 and 532_02, but not against 523_04 and 531_01. These two R-genes are clearly race-specific rather than of quantitative nature.

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Identifying loci involved in the pollen rejection response at the stigma surface in perennial ryegrass – update and progress

Daniel Thorogood[a], Chloe Manzanares[b], Steven Yates[b] and Bruno Studer[b]

[a] *Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, United Kingdom*
[b] *Forage Crop Genetics Group, Institute of Agricultural Sciences, ETH Zurich, Switzerland*

dnt@aber.ac.uk

The first physiological barrier to successful fertilisation of ovules by pollen gametes in higher plants occurs at varying points along the stylar transmission tract. Several diverse mechanisms have been described in a number of families. The response usually results in rejection of genetically similar individuals (self-incompatibility) and is a mechanism that prevents selfing and consequent inbreeding depression in around half of all flowering plants. Self-incompatibility occasionally breaks down to allow self-seed to be set. In addition, apparently physiologically identical mechanisms are involved in rejection of pollen from related species that prevent inter-specific hybridisation. Quite often rejection only occurs in one direction and is known as unilateral incompatibility.

In perennial ryegrass, and in common with all grasses studied, two loci, *S* and *Z*, act complementarily to reject pollen when both *S* and *Z* alleles are matched in pollen and stigma. The mechanism is under gametophytic control, i.e. the response is dependent on an interaction between individual pollen grains and the receptive female organs. The reaction is immediate: activation is at the stigma surface where pollen grains alight. Self-fertility can occur: both *S* and *Z* have self-fertile allelic forms and a number of additional modifier loci also exist.

We are using genetic mapping and association mapping studies to identify the genes responsible. To date we have identified previously unknown genes co-segregating with *S* (Manzanares et al, 2016) and *Z* (in preparation). We summarise knowledge to date and, we also present the results of a genome wide association study (GWAS) that powerfully confirms the existence of a suite of compatibility loci in a single breeding population. *In-vitro* pollinations were made in a full diallel and compatibility scores were used to produce similarity matrices that were subjected to principal co-ordinates analysis in order to simplify the compatibility relationships of the 52 representative individuals of the population. Scores for the first four principal components were then subject to genome-wide association analysis using a custom ryegrass Illumina chip array containing 3,800 gene-based SNP markers of which 2,478 had been mapped using a pair-cross-based mapping family and 704 were unmapped. A number of known *S* and *Z* gene markers were also included in the analysis. The markers with the highest significance were those closest to the known positions of *S* and *Z* on chromosomes 1 and 2 based on comparative map positions with model grass species, rice and *Brachypodium distachyon*. Markers on chromosomes 3 and 6, which may well coincide with loci from other studies that are known to influence the self- and cross-incompatibility status of ryegrass plants, were also significantly associated.

Our research to date demonstrates the potential of mapping and association mapping techniques for identifying pollen-stigma compatibility genes. Identifying these genes will enable the characterisation and potential manipulation of the extremely rapid pollen-stigma interaction process that determines the initial fate of pollen, intent on fertilisation.

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Parallel oral presentations: Maize and sorghum

The study of cytoplasmatic diversification role on some productivity elements on maize

Roxana E. Calugar[a, b], Voichita V. Has[a], Andrei Varga [a,b], Carmen D. Vana [a], Ana Copandean [a] and Ioan Has [a,b]

[a] *Agricultural Research and Development Station Turda, Romania*
[b] *University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania*

roxasut@yahoo.com

Cytoplasmic male sterility discovery has led many researchers to move their studies towards cytoplasmic diversification, extrachromosomal heredity and the influence the cytoplasm can have on inheriting some important agronomic traits in hybrids. Assuming that among the various sources of cytoplasm there could be some differences in their genetic value, at ARDS Turda there was created some isonuclear inbred lines, by using the backcross for 10 years. After backcrossing, it was estimated that the paternal nucleus was transferred 99.9% in the cytoplasm of the donor inbred line.

This paper presents the results of genetic studies of five groups of isonuclear inbred lines created by transferring the nucleus of the elite inbred TC 209, TC 316, TC 243, TB 367 and D 105 on the cytoplasm of T 248, TB 329, TC 177 and TC 221 inbreed lines. The testing of the isolines was done by crossing each of the 25 inbred lines with four testers. Isonuclear lines test fields have been studied in two experimental years (2013-2014), being carried out biometrics on some characters of the cobs.

Between the studied cytoplasms there were no significant differences in grain yield of the plot, ear weight, grain weight per ear, ear length and number of kernels per row, but these characters were influenced in some cases by cytoplasmic nuclear interaction.

Genetics of hybrid performance in maize: QTL detection for biomass production in a reciprocal multiparental design

Héloïse Giraud[a], Cyril Bauland[a], **Alain Charcosset[a]** and Laurence Moreau[a]

[a] *INRA, UMR Génétique Quantitative et Evolution, Gif sur Yvette, France*

charcos@moulon.inra.fr

Understanding genetic architecture of hybrid performances is of key importance for allogamous species such as maize (Zea mays L.). We developed two multiparental populations corresponding each to one of the main heterotic groups used for maize silage production in Northern Europe (the dent and flint groups). In each group, four founder lines were crossed to produce six connected biparental populations of segregating lines. These lines (821 and 801 for the dent and flint group, respectively), were genotyped for approximately 20k SNPs and were crossed according to an incomplete factorial design to produce 951 dent-flint hybrids, evaluated for silage performances in eight environments. Hybrid genetic variance decomposition showed a predominance of general (GCA) over specific (SCA) combining abilities. SCA explained between 13.8 and 22.6% of the within-population hybrid variance, depending on the trait. QTL detection was carried out for GCA and SCA using different models considering allelic effects transmitted from each founder lines (linkage analysis) or considering directly SNP alleles (linkage disequilibrium mapping) assuming equal or different effects in each group. In total, between 42 and 54 QTLs were detected depending on the model, among which 12 to 31% presented dominance/SCA effect significant at a 5% individual risk level. Only 16 QTL were detected by all three models illustrating their complementary. Most of the QTL (about 80%) were specific to one group, consistent with the long term divergence between the dent and the flint group. These results open interesting prospects for revisiting with markers the concept of reciprocal recurrent selection.

This study was conducted in the frame of Promaïs project SAMMCR, involving INRA, Caussade Semences, Euralis Semences, Limagrain Europe, Maïsadour semences, Pioneer Genetics, R2n and Syngenta Seeds.

Fine mapping and cloning of a major QTL for flowering time on maize chromosome 3

Francesco Emanuelli[a], José M. Soriano[a], Linda Zamariola[a], Silvia Giuliani[a], Riccardo Bovina[a], Danara Ormanbekova[a], Rachil Koumproglou[b], Torben Jahrmann[b], **Roberto Tuberosa[a]** and Silvio Salvi[a]

[a] *Department of Agricultural Sciences, University of Bologna, Viale Fanin 44, 40127, Bologna, (Italy)*
[b] *Semillas Fitó S.A., Selva de Mar 111, 08019, Barcelona (Spain)*

roberto.tuberosa@unibo.it

Flowering time is a complex trait important for crop adaptation to the local environment. A major quantitative trait locus (QTL) for flowering time and number of nodes (ND), *qVgt3.05*, was previously identified on chromosome 3, bin 3.05, in a maize introgression library (IL) population derived from the cross B73 x Gaspé Flint (recipient and donor genotypes, respectively. Salvi et al. 2011). In this region, other major flowering time QTLs have previously been mapped, thus underlying the importance of this locus. In order to fine map and to clone this *QTL*, one line (39-1-2-33) was derived from the IL populations, being early flowering (~17 ND) compared to B73 (~20 ND) and carrying only a small Gaspé Flint introgression (17-cM) on chromosome 3 that includes *qVgt3.05*. From the cross between B73 and line 39-1-2-33, 3348 F2 plants were derived, genotyped, and phenotyped for ND. QTL mapping placed *qVgt3.05* within a 0.3 cM interval; in this cross the QTL showed an addictive effect of 1,36 nodes and explained 56.6% of the phenotypic variance. For positional cloning, a total of 7500 F2 plants were phenotyped for ND and genotyped with SSR markers flanking the QTL interval. One-hundred F2 recombinants were identified and F3 and/or F4 recombinant families were derived and grouped into seven classes based on the type of recombination events around the QTL. Phenotypic and molecular analysis of these lines enabled to further narrow the target genomic region to a 480-kb interval. Two putative candidates, a MADS-box gene and a Squamosa binding protein-transcription factor gene, mapped within this region. The role of the two candidate genes is currently under study using several approaches, including comparison of allelic sequences and quantitative profiles of gene expression between the two parental haplotypes B73 and Gaspé Flint and including selected F3:4 isogenic lines as well.

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Mining novel alleles for maize provitamin A enrichment and product delivery

Abebe Menkir[a], Bussie Maziya-Dixon[a], Oladeji Alamu[a] and Torbert Rocheford[b]

[a] *International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria*
[a] *Department of Agronomy, Purdue University, West Lafayette, Indiana, USA*

a.menkir@cgiar.org

Millions of people in Africa subsist on cereal-based diets with low levels of vitamin A and run a high risk of vitamin A deficiency, which causes developmental disorders, impairs eyesight, and weakens immune system essential for warding off debilitating disease. Biofortification of maize with provitamin A has thus been considered a long-term approach to improve vitamin A status of human populations with unbalanced diets. As the most commonly grown and consumed yellow maize cultivars in Africa contain less than 2 μg/g provitamin A, 12 exotic lines were introduced as potential sources of favourable alleles to further boost the concentrations of ß-carotene and provitamin A in tropical maize inbred lines. The backcrosses containing these donor lines have been sources of advanced maize inbred lines with bright yellow to orange kernel colour and semi-flint to flint kernel texture as well as desirable agronomic and adaptive traits. Many of the backcross-derived lines contain ß-carotene concentrations varying form 5.0 to 20.0 μg g^{-1} and pro-vitamin A content varying from 8.0 to 22.3 μg g^{-1} in their kernels. Several of these lines carry the favourable alleles of the most significant functional markers of crtRB1-3T' and crtRB1-5'TE derived from the exotic lines that were absent in adapted recurrent parents. Many of these maize inbred lines have been used to develop hybrids and synthetics dispatched to partners for extensive regional testing. Recent trial results found some synthetics and hybrids with 7.0 to 12.8 μg/g of provitamin A that are also competitive with the most commonly grown orange maize cultivars in terms of grain yields and other agronomic traits. This testing scheme was the vehicle for selection and release of the first generations of hybrids and synthetic varieties with intermediate levels of provitamin A in Ghana, Mali, and Nigeria.

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The role of intra-crop competition in efficiency of resource use and breeding

Ioannis Tokatlidis

Department of Agricultural Development, Democritus University of Thrace, Greece

itokatl@agro.duth.gr

Modern agriculture is faced with a double contrasting challenge, the demand for higher food productivity versus the enormously fluctuating environment that hampers productivity. A major problem that should be addressed urgently is a considerable yield gap (potential vs obtainable yield). Competition among plants within the crop stand, either genetically or environmentally induced, constitutes a particular root cause for this gap. From the agronomy perspective, competition pertains to inequality among individuals connected with unequal use of the limited growth sources. The intensity of competition is reflected by the plant-to-plant differences defining the magnitude of the competitive advantage of some individuals over others. Since inter-plant competition conditions the effectiveness in resource capture, appraising the kind of competition that prevails in different cropping systems is essential. The acquired part of competition (i.e., the environmentally induced) is always present, depending on crop management and genotype vulnerability to abiotic and biotic forces. Genetically induced competition exists in intra-species and/or inter-species genetic differences, by implication expanding the acquired competition as well. In terms of a sole crop of a mono-genotypic variety, only acquired competition prevails, while intra-species genetic competition is added in a sole crop of a multi-genotypic variety. In inter-cropping systems, the inter-species genetic competition occurs on the premise that only mono-genotypic varieties are mixed, while both intra- and inter-species competition is present when multi-genotypic varieties are mixed. Thus theoretically, the lowest competition is found in sole crops of mono-genotypic varieties and the highest when multi-genotypic varieties are mixed. Involvement of the appropriate variety(ies) in a particular kind of agricultural system is of prime importance so as to mitigate the intra-crop competition and optimize resource use efficiency and production. From a breeding perspective, intra-species competition exerts a confounding influence on selection because of the negative relationship between genotype competitive and yielding ability. Hence, breeding at minimum competition (widely apart plants to preclude plant-to-plant interference for inputs) is a necessary condition to develop ‘specific’ varieties capable of satisfying the demands of the various cropping systems. Breeding at minimum competition is asserted as ideal to breed varieties devoid of insatiable individuals that withstand acquiring competition and mitigate genetic competition. Further, thanks to focusing on single-plant performance, the varieties are distinguished for density-independence. Density-independent varieties can be cultivated at lower densities than commonly used, an asserted essential prerequisite for effective use of resources, over-season stability to cope with the fluctuation of the environment, and to bridge the yield gap. This agronomic trait matches the basic rule of crop spacing in the innovative ‘system of crop intensification’ invented for low-input agriculture.

Paving the way towards the development of biomass sorghum: a transdisciplinary approach for the development of new sorghum varieties

Ingrid Vilmus[1], Gilles Trouche[1], Nicolas Le Moigne[2], Hélène Carrère[3], Delphine Luquet[1], Denis Bastianelli[4], Patrice Jeanson[5], Joël Alcouffe[6], Patrick Navard[7], Korotimi Thera[8], Niaba Témé[8], Yves Barrière[9], Herman Höfte[9] and David Pot[1]

[1]UMR AGAP, Cirad, France ; [2] Centre des Matériaux des Mines d'Alès, Ecole des Mines d'Alès, France ; [3] Laboratoire de Biotechnologie de l'environnement, INRA Narbonne, France ; [4] UMR SELMET, Cirad, France ; [5] Euralis Semence, France ; [6] RAGT2n, France ; [7] Centre de Mise en Forme des Matériaux, Ecole des Mines de Paris, France ; [8] Centre Régional de Recherche Agronomique de Sotuba, Institut d'Economie Rurale, Mali ; [9] Institut Jean-Pierre Bourgin, INRA Versailles, France

ingrid.vilmus@cirad.fr

Plant biomass is expected to become an essential source to substitute fossil Carbon used currently for energy and biomaterials. As a C4 grass, Sorghum has an efficient photosynthesis which results in a high biomass production and is a promising candidate species for the development of biomass value chain. To reach this objective a transdisciplinary approach merging material sciences, histology, biochemistry, physiology, modeling, genetics and breeding is being developed.

Firstly, biomass traits affecting the properties of different end-products were identified through the combined efforts of materials scientists, process developers and geneticists. We showed that high cellulose content combined with low biomass digestibility is required for polymer biocomposites in order to ensure their thermal and mechanical resistances. At the opposite, this combination of traits is negatively linked to the methane production potential.

Secondly, on some contrasted genotypes, the traits of interest where characterized all along the sorghum development in different water regimes in order to identify their patterns of accumulation / degradation and their response to abiotic constraints. Transcriptome analysis was also performed to clarify the gene regulatory network underlying these traits. Relationships between the biomass production and biomass quality related traits were explored through a modeling approach. It allowed studying the relevance of different ideotypes according to the crop management and environmental constraints.

Thirdly, the genetic determinism of the traits of interest was explored in order to optimize the breeding efficiency for the development of new ideotypes. Allele discovery in broad based panels aiming to build a general library of genomic regions of interest was performed simultaneously with the development of more specific breeding schemes dedicated to the analysis of particular traits and breeding optimization for specific ideotypes. Three connected broad-based association panels were phenotyped for biomass traits highlighted in previous steps. Various trials encompassing different years and locations (tropical or temperate) were analyzed through GWAS analysis using a large SNP dataset. Some promising candidate genes were identified taking advantage of a comparative genomic approach with other species. As a complementary approach, dedicated breeding designs for bioethanol, methane or biocomposites production were used to optimize the identification of the genomic regions of interest and develop new elite parental lines.

We expect that the use of this transdisciplinary approach will provide the sorghum community with relevant generic molecular tools and elite parental lines to monitor sorghum ideotype development adapted to the different uses.

Fine mapping and characterization of JAT, a major locus regulating the transition from juvenile to adult phase in maize

Linda Zamariola[a], Francesco Emanuelli[a], José Miguel Soriano[a], Riccardo Bovina[a], Silvia Giuliani[a], Danara Ormanbekova[a], Rachil Koumproglou[b], Torben Jahrmann[b], Roberto Tuberosa[a] and Silvio Salvi[a]

[a] *Department of Agricultural Sciences, University of Bologna, Italy*
[b] *Semillas Fitó S.A., Barcelona (Spain)*

linda.zamariola2@unibo.it

Maize is not only an important crop for grain and forage production but is also one of the most valuable energy crops for biofuel needs. Triggered by different internal and external stimuli, maize goes through a series of three main developmental stages over time, namely juvenile vegetative, adult vegetative, and reproductive. The developmental transition that mostly influences biofuel production is the switch from juvenile to adult phase of development (JAT). In fact, previous analysis of juvenile biomass in maize have shown that it possesses decreased lignin and increased levels of certain sugars, making it a superior substrate for fermentation by reducing the recalcitrant biomass to enzymatic saccharification. The JAT transition generally takes place at leaf 6-7 and can be easily identified by observing the disappearance of epicuticular waxes, the development of hairs and the formation of a thick cuticle.

In this work we report the fine mapping of a locus governing JAT in maize. A B73-nearly isogenic line (39-1-2-33) from a Gaspé Flint/B73 introgression library (Salvi et al. 2011, BMC Plant Biol, 11, 4) showed a prolonged juvenile phase (transition approximately at leaf 9 compared to leaf 6-7 of B73). A B73 x 39-1-2-33 F_2 population of more than 4000 plants was genotyped with SSR and de novo SNP markers and phenotyped by marking the leaf showing the transition between juvenile and adult phase. The fine mapping narrowed down the QTL to a 0,16 cM region corresponding to a 400 kb genomic interval. This region encompasses very few candidate genes potentially involved in promoting juvenile leaf traits. Validation and characterization of the putative functional candidate genes is ongoing by gene expression analysis and sequencing of the Gaspé Flint haplotype. Furthermore, the biomass chemical composition of the 39-1-2-33 line in comparison to B73 is being evaluated.

Parallel oral presentations: Vegetables

Genome-wide study of the tomato *SlMLO* gene family and its functional characterization in response to the powdery mildew fungus *Oidium neolycopersici*

Michela Appiano [a], Zheng Zheng [b], Stefano Pavan [c], Valentina Bracuto [c], Luigi Ricciardi [c], Richard G.F. Visser [a], Anne-Marie Wolters [a] and **Yuling Bai [a]**

[a] *Wageningen UR Plant Breeding, Wageningen University and Research Centre, The Netherlands*
[b] *Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, China*
[c] *Department of Plant, Soil and Food Science, Section of Genetics and Plant Breeding, University of Bari Aldo Moro, Italy*

bai.yuling@wur.nl

The *MLO* (Mildew Locus O) gene family encodes plant-specific proteins containing seven transmembrane domains and likely acting in signal transduction in a calcium and calmodulin dependent manner. Some members of the *MLO* family are susceptibility genes (S-genes) towards fungi causing the powdery mildew disease (Consonni et al., 2006).
In tomato, for example, the loss-of-function of the *MLO* gene *SlMLO1* leads to a particular form of powdery mildew resistance, called *ol-2*, which arrests almost completely fungal penetration (Bai et al., 2008). This type of penetration resistance is characterized by the apposition of papillae at the sites of plant-pathogen interaction. Other *MLO* homologs in Arabidopsis regulate root response to mechanical stimuli (*AtMLO4* and *AtMLO11*) and pollen tube reception by the female gametophyte (*AtMLO7*) (Chen et al., 2009; Kessler et al., 2010). However, the role of most *MLO* genes remains unknown.
In this work, we provide a genome-wide study of the tomato *SlMLO* gene family. Besides *SlMLO1*, another fifteen *SlMLO* homologs were identified and characterized with respect to their structure, genomic organization, phylogenetic relationship, and expression profile in axenic conditions and upon pathogen challenge. In addition, by analysis of transgenic plants, this study shows that simultaneous silencing of *SlMLO1* and two of its closely related homologs, *SlMLO5* and *SlMLO8*, confer a higher level of resistance than the one associated with the *ol-2* mutation. This finding provides evidence for functional redundancy among tomato homolog genes involved in powdery mildew susceptibility.
Moreover, since breeding for resistance by loss-of-function of S-genes represents an important strategy to achieve durable and broad-spectrum resistance, this study is particularly interesting to accelerate research translation to other crops affected by the powdery mildew disease (Pavan et al., 2010). The recommendation when searching for *MLO* susceptibility genes is to select candidates from clade IV or V (in case of a monocot or a dicot species, respectively) that are induced by PM infection. However, the observed upregulation of tomato *SlMLO* genes outside clade V in response to PM raises the possibility that they may also act as susceptibility genes. Finally, during this study a series of transgenic lines silenced for individual *SlMLO* homologs were developed, which lays the foundation for further investigations aimed at assigning new biological functions to the *MLO* gene family.

A high quality eggplant genome sequence: a new tool for the analysis of Solanaceae family evolution and for the molecular deciphering of complex traits.

Lorenzo Barchi[a], Massimo Delledonne[b], Sergio Lanteri[a], Alessandra Dal Molin[b], Andrea Minio[b], Alberto Ferrarini[b], Luca Venturini[b], Avanzato Carla[b], Laura Toppino[c], Tea Sala[c], Laura Bassolino[c], Cinzia Comino[a], Alberto Acquadro[a], Ezio Portis[a], Riccardo Rinaldi[a], Davide Scaglione[a], Gianluca Francese[d], Antonietta D'Alessandro[d], Giuseppe Mennella[d], Domenico Perrone[d], Nazzareno Acciarri[e]., Marco Pietrella[f], Giuseppe Aprea[g], Maria Sulli[g], Giovanni Giuliano[g] and Giuseppe L. Rotino[c]

[a] *DISAFA, Plant Genetics and Breeding, University of Torino, Italy*
[b] *University of Verona, Italy*
[c] *CREA-ORL, Montanaso Lombardo, Italy*
[d] *CREA-ORT, Pontecagnano, Italy*
[e] *CREA-ORA, Monsampolo del Tronto, Italy*
[f] *CREA-FRF, Forli, Italy*
[g] *ENEA, Casaccia Research Center, Roma, Italy*

lorenzo.barchi@unito.it

Eggplant (*Solanum melongena* L. 2n = 2x = 24) is the third most important Solanaceous crop, after potato and tomato, with a worldwide production of about 49.5 Mt in 2013 (http://faostat.fao.org), being Italy the first European producer.
Tomato and potato are closer to each other and belong to the subgenus Potatoe, while eggplant to the subgenus Leptostemonum, thus representing a unique member for comparative genomic analyses within the genus *Solanum.* The Solanaceae family also includes the most distantly related pepper, a member of the genus *Capsicum.*
We produced an high quality reference genome by Illumina sequencing (155 X) of the inbred eggplant line '67/3', which is the male parent of a RIL mapping population of 157 F6 progeny. The draft assembly spans ~1.2 Gb (L50 of > 640kb), of which over 900 Mb were covered by Illumina contigs. By applying an high-resolution restriction map, based on Bionano Genomics optical mapping technique, the fragmentation of the hybrid assembly was further reduced (L50 of > 3Mb). Following Illumina sequencing (35X) of the line '305E40' (female parent) and the RILs (1X), the hybrid assembly was assigned to 12 pseudomolecules on the basis of linkage analyses.
The availability of eggplant, tomato, potato and pepper genome sequences provided a tool to deduce contiguous ancestral regions (CARs) within the Solanaceae family. The latter experienced a genomic triplication about 50MYA ("T" event), resulting in the presence of genes which still remain triplicated/duplicated.
The CoGe platform (https://genomevolution.org/coge/) was applied to identify unique orthologous genes among the four species, and coffee was used as outgroup. CARs at pepper (Solanaceae), eggplant (Solanum) and tomato/potato (Potatoe) evolutionary divergence as well as after speciation were reconstructed. This highlighted the features and outcomes of chromosomal rearrangements arose during Solanaceae evolution, and which occurred at higher rate in certain chromosomes than others. The mapping population of 157 RILs was extensively phenotyped for metabolic as well as for key fruit (shape and size) and plant traits and related QTLs detected.

Developing methods to assess and quantify abiotic stress responses in *Brassica oleracea* and tipburn tolerance in lettuce (*Lactuca sativa*)

Andrew M. Beacham, James M. Monaghan, Paul Hand and David A. C. Pink

Fresh Produce Research Centre, Crop and Environment Sciences Department, Harper Adams University, UK

abeacham@harper-adams.ac.uk

Leafy vegetable crops include *Brassica oleracea*, varieties of which represent familiar foods such as cabbage, cauliflower, broccoli and kale, and lettuce (*Lactuca sativa*), one of the most commercially important salad crops globally. Changing climatic conditions are placing constraints on agricultural production and as such, breeding to provide new lines with increased tolerance of abiotic stresses and their associated physiological conditions (such as lettuce leaf margin discolouration or 'tipburn') is required. The development of pre-breeding genetic resources such as the Vegetable Genetic Improvement Network (VeGIN) *B. oleracea* and *Lactuca* sp. Diversity Fixed Foundation Sets (DFFSs) and mapping populations provides the opportunity to identify new sources of genetic material for downstream breeding programmes. We have therefore developed assays to analyse the response of lines of the VeGIN *B. oleracea* and *Lactuca* sp. DFFSs to a range of abiotic stresses and tipburn-inducing conditions, respectively. Screening 72 *B. oleracea* DFFS lines for tolerance to drought, salinity and flooding stresses identified a number of lines exhibiting tolerance of one, two or all three stresses. Selected lines are now being analysed for their response to heat and cold stresses. For 96 *Lactuca* DFFS lines, using a hydroponic system with reproducible tipburn induction, we found lines belonging to both the cultivated species *L. sativa* and the wild relatives *L. serriola*, *L. saligna* and *L. virosa* which appeared to show low susceptibility to tipburn development. In addition, we found differential responses of the two parents of the VeGIN *L. sativa* mapping population in the tipburn assay. We are now screening this population in both glasshouse and field conditions in order to identify QTLs conferring tipburn tolerance that can be used for future marker-assisted breeding programmes.

We wish to thank the University of Warwick Crop Centre Vegetable Genetic Improvement Network (VeGIN) Team for their assistance. This work is funded by the UK Government Department for Environment, Food and Rural Affairs (DEFRA).

A genetic approach to improving postharvest quality in lettuce

Jim Monaghan[a], Paul Hunter[a], Dave Pink[a], Guy Barker[b], Stella Lignou[c] and Carol Wagstaff[c]

[a] *Department of Crop and Environment Research, Harper-Adams University, UK*
[b] *School of Life Sciences, University of Warwick, UK*
[c] *Department of Food Nutritional Sciences, University of Reading, UK*

jmmonaghan@harper-adams.ac.uk

'Ready to eat' products have considerable added value e.g. UK lettuce has a farm-gate value of £266m while the retail value of UK processed salads is estimated to be £800m. However, such products have increased perishability resulting in high wastage (~1.36m tonnes with a value of £2.39m annually with nearly 75% due to loss of quality). Many leafy vegetables and fruits are susceptible to discolouration and this is a major reason why fresh produce fail to meet shelf life targets

Modified atmospheric packaging (MAP) (essentially, the removal of oxygen) can delay post-harvest discolouration and prolong shelf life, however, it increases production costs, requires specialised equipment and optimization for specific products. In addition, MAP does not prevent discolouration once the pack has been opened.

Breeding crop varieties with reduced propensity to discolour offers a cost effective solution as growing them has no added costs. This project facilitates a genetic approach to controlling post-harvest discolouration by developing an understanding of the genetics and biochemistry of discolouration and providing underpinning knowledge to allow exploitation of quantitative natural variation in the development of discolouration whilst maintaining other traits (e.g. disease resistance, taste etc.) at acceptable levels.

The approaches being used within the project to gain greater understanding of the genetic and biochemical regulation of post-harvest discolouration (pinking and browning will be outlined, including field trials and subsequent phenotypic, metabolic and molecular analyses and controlled environment experiments.

Key results from the first year include demonstration of significant variation for post-harvest pinking and browning in recombinant inbred lettuce population derived from a cross between cultivars Saladin x Iceberg, evidence to suggest that the two symptoms have different genetic controls, identification of significant QTL for both pinking and browning phenotypes and levels of variability in both key gene sequences and metabolic activities associated with the biochemical pathways thought to be involved. Future planned work including further field trials with the lettuce genetic diversity set and transcriptome studies will be also be described.

Determination of genes involved in drought mechanism in melon genetic resources

Ayse Ozer[1], Nebahat Sari[2], Yıldız Aka Kacar[2] and Ozhan Simsek[2]

[1]Faculty of Agriculture, Selcuk University, Konya, Turkey
Faculty of Agriculture, Department of Horticulture, University of Cukurova, Adana, Turkey

aozer@selcuk.edu.tr

Global climate change and environmental factors such as drought are limiting melon yield in recent years. High drought tolerant genetic material can be found in Turkey which has rich genetic diversity. In this research we determined the levels of drought tolerance in melon genotypes; both in the climate-controlled greenhouse and in water culture at climate-controlled chamber. In the pre-screening step of this work, we have used 192 genotypes. In the second year we have used 20 melon genotypes (10 drought-tolerant and 10 sensitive). Drought was applied by mixing PEG 6000 into Hoagland solution. In control only Hoagland solution was used. Within 192 genotypes, the most tolerant genotypes to drought was Kav-248 and the most sensitive genotype was Kav-20. Leaf and root samples were taken from the control group and drought treated group at 0, 4, 8, 12, 24, 72 hours in the selected genotypes. Leaf and root samples were frozen in liquid nitrogen and stored at -85^{o} C for molecular analysis. RNA was isolated from leaves and roots from the genotypes. mRNA isolation was made from total RNA and later on cDNA synthesis was realized. Expressed genes were identified by cDNA-AFLP method in case of drought stress. EcoRI and Msel were used as a cutting enzyme at cDNA-AFLP analysis. Selective PCR reactions were realized using 77 primary combinations after pre-amplification. PCR products were run in 6.5% polyacrylamide gel. The PCR products in acrylamide gel were examined and DNA profiles at different levels among samples were cut from the gel. These DNA profiles were amplified again. At different levels, DNA sequencing of expressed transcripts were conducted. Functions of genes and proteins were searched at NCBI data bank. As a result of this study, genes related to drought stress based on the expression analysis were detected and discussed.

Perspectives on cucurbit crop history

Harry S. Paris

Newe Ya'ar Research Center, Agricultural Research Organization, Israel

hsparis@volcani.agri.gov.il

The gourd family, Cucurbitaceae, has furnished a number of vegetable crops. The five most widely familiar cucurbit crops are cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), watermelon (*Citrullus* spp.), and squash and pumpkin (*Cucurbita* spp.). Records of the use of cucurbits by people take the form of archaeobotanical remains, iconography and literature. Cucumber and melon derive from Asia, watermelon from Africa, and squash and pumpkin from the Americas. Melons and cucumbers were probably intially cultivated for the use of their young fruits as vegetables. Melons spread to eastern Africa at an early date, by 4000 years ago, but cucumbers are probably a more recent domesticate and spread westward later, reaching Europe in early medieval times. The earliest records of sweet melons date to early medieval times in Khorasan, Central Asia. Dessert watermelons, *Citrullus lanatus* (Thunb.) Matsum. & Nakai, were cultivated in northeastern Africa at least 4000 years ago, first probably as a source of fresh water. Sweet dessert watermelons have been recorded since the second century CE. *Cucurbita* spp. were first cultivated in the Americas. Remains of domesticated *Cucurbita pepo* L. in North America and *Cucurbita moschata* Duchesne in South America date to 10,000 years ago. By 1492 a number of cultivar-groups of pumpkins and squash of *Cucurbita pepo* had been developed by indigenous American peoples. Interestingly, though, the zucchini squash, the cultivar-group of *Cucurbita pepo* that has by far the most monetary value, is a recent development, traceable to nineteenth-century northern Italy. Most wild cucurbits, for example wild melons, watermelons, and *Cucurbita* spp., have round or oval fruits. Although the cultivated cucurbits derived from different genera have a wide range of culinary uses, there are some parallels among them. Cucurbit cultigens that are grown for culinary use of the young fruits share deviation from ancestral roundness and 1:1 length-to-width ratio of the fruits. In most cases, the fruits are noticeably elongated and in others noticeably flattened. Cucumbers, summer squash, sponge gourds (*Luffa* spp.), snake gourds (*Trichosanthes cucumerina* L.), and the edible-fruited bottle gourds (*Lagenaria siceraria* (Mol.) Standl.) are grown for consumption of the young fruits, which deviate greatly from 1:1 length-to-width ratio and roundness. People selected for deviation from roundness in order to obtain fruits which had a higher proportion of colored exocarp and firm mesocarp, and less seedy endocarp. In contrast, most cultigens of cucurbits grown for consumption of the mature fruits, including watermelons, melons, winter squash, pumpkins, and wax gourds (*Benincasa hispida* (Thunb.) Cogn.), are round or at least do not deviate greatly from a 1:1 length-to-width ratio. Melons (*Cucumis melo*), and squash and pumpkins (*Cucurbita* spp.), which have many cultigens that are consumed immature and many others mature, are the most polymorphic of the cucurbits.

The *Fom-1-Prv* pair of melon resistance genes: lessons from expression and interaction studies

Arie Amitzur, Golan Miller, Michael Normantovich, Amalia Bar-Ziv, Katya Pashkovsy and **Rafael Perl-Treves**

Faculty of Life Sciences, Bar Ilan University, Ramat Gan 5290002, Israel

rafi.perl@gmail.com

NBL (nucleotide binding site–leucine rich repeat) encoding genes are the prevalent class of resistance genes. Their products recognize pathogen effectors (*Avr* factors) and provide plants with a dynamically evolving system to monitor pathogen invasion. Their mapping, cloning and functional characterization in crop species provide important tools for plant protection. We have cloned the genomic locus for melon resistance towards two pathogens, *Fusarium oxysporum* f.sp. *melonis* (FOM) races 0 and 1, and papaya ring spot virus (PRSV). The two adjacent *Prv* and *Fom-1* genes encode proteins of the NBL family. *Prv* carries an extra NB domain, and both genes have alternative, differentially expressed splice variants. Paired R-genes were recently suggested to function together in a novel cooperative mechanism, and we are exploring this possibility for *Prv* and *Fom-1*. The genes' expression patterns were studied using a promoter-reporter system in transgenic melon roots using a "composite plant" system. Endogenous transcripts were also quantified by RT-PCR, to evaluate possible co-expression and pathosystems, we looked for host-pathogen protein interactions. In a complementary study, we analyzed the proteome of the xylem sap of melon plants infected with FOM-0. A total of 513 melon protein and ~40 fungal proteins could be annotated against the melon and FOM genomes, respectively. Many of the melon proteins appear to be induced by inoculation, providing interesting defense gene candidates. Among fungal proteins, candidate *Avr* factors could be suggested.

Parallel oral presentations:
Fruit, ornamentals, medicinal/aromatic plants

Accelerated introgression of wild apple Fire blight resistance originating from *Malus* x *robusta* 5 by the method "Fast Track"

Simone Schütz[a], Isabelle O. Baumgartner[a], Andreas Peil[b] and Markus Kellerhals[a]

[a] *Institute for Plant Production Sciences IPS, Agroscope, Switzerland*
[b] *Institute for Breeding Research on Fruit Crops, Julius Kühn-Institut (JKI), Germany*

simone.schuetz@agroscope.admin.ch

Breeding for high fruit quality, durable disease resistance and yield security is an important contribution to ecological intensification in apple growing. Since 1985, the apple breeding programme at Agroscope in Wädenswil is focused on breeding for resistances against several major diseases (scab: *Venturia inaequalis*, powdery mildew*: Podosphaera leucotricha*, fire blight*: Erwinia amylovora*, etc.). Disease resistance is combined with high fruit quality, good storage and high yielding capacity. The use of fire blight resistant cultivars in combination with an integrated control management is a promising control strategy (Baumgartner et al. 2014). However, currently only a few partially resistant cultivars are ready for the market (e.g. 'Ladina', Kellerhals et al., 2011). Strong fire blight resistances are found in the natural genetic diversity of wild *Malus* species, e.g. *M.* × *robusta* 5 (MR5), *M. baccata*, *M. fusca* (Peil et al. 2009; Emeriewen et al. 2014). However, fruit size and quality of wild resistance genitors are insufficient and not comparable with modern apple cultivars available on the market. In order to achieve commercial quality combined with fire blight resistance from a wild species, several pseudo-backcrosses with high quality parents are required. To accelerate the long generation cycle of *Malus*, usually 4 to 5 years, a low-input "Fast Track" breeding approach under greenhouse conditions was tested and continuously improved. Seedlings from the pseudo-backcrosses are screened with molecular markers for the presence or absence of the fire blight resistance (FB_MR5, Fb_E, Fb_Mfub10). Additionally, the presence of several other markers for scab and/or powdery mildew resistance is tested to identify resistances expected according to the pedigree. The selected seedlings are grown in pots on their own roots in the glasshouse under optimal growing conditions to enhance flowering, including non-limiting irrigation and fertilisation, regular prohexadione-Ca and ethephon treatments to induce compact growth and to simulate the end of the vegetation period, respectively, followed by artificial winter simulation in a cold store. All these measures stimulate the transition of the seedling from vegetative to generative growth. Pollen is collected from flowering plants for crosses with high quality cultivars and flowers are pollinated with pollen from elite apple germplasm. Parents for new introgression cycles are selected based on fruit evaluation and on results of artificial shoot inoculation tests with a Swiss strain of *Erwinia amylovora* in the quarantine greenhouse to evaluate phenotypic fire blight susceptibility.

The latest results of the F3 and F4 generation with FB_MR5 resistance will be shown and discussed, including results of artificial shoot inoculation, population studies, breeding achievements and fruit quality development.

Innovative strategies towards marker-assisted pre-breeding for Downy and Powdery mildew resistance in grapevine

Elisa Peressotti[a], Chiara Dolzani[a], Elisa Banchi[a], Lara Poles[a], Daniele Buonassisi[a,b], Daniele Migliaro[c], Elena Arrigoni[a], Antonella Vecchione[a], Luca Zulini[a], Eric van de Weg[d], Marco C.A.M. Bink[e], Marco Stefanini[a], Riccardo Velasco[a] and **Silvia Vezzulli**[a]

[a] *Research and Innovation Centre, Fondazione Edmund Mach, Italy*
[b] *Department of Agricultural and Environmental Sciences, Università degli Studi di Udine, Italy*
[c] *Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia agraria - Centro di Ricerca per la Viticoltura (CRA-VIT), Italy*
[d] *Department of Plant Breeding, Wageningen UR, The Netherlands*
[e] *Biometris, Wageningen UR, The Netherlands*

silvia.vezzulli@fmach.it

Grapevine (*Vitis vinifera* L.) is one of the most valuable crops worldwide, mainly studied for quality and disease resistance traits. Viticulture has often been affected by encounters with new parasites that still represent a major constraint. This is a particularly important issue because, even though some inter-specific varieties (hybrids) between *V. vinifera* and *Vitis* spp. are widely present, the majority of cultivated grapevines are pure *V. vinifera* varieties, which are highly susceptible to pathogen attack. Fungal diseases represent some of the most severe plagues and growers are obliged to use pesticides to prevent serious yield loss.

Within the FEM grapevine breeding program, re-established in the middle '80s, the selection process has been based on the major need for innovation raised by grapevine growers. During the past years, this request has been addressed to increase the complexity and the originality of wines, while in the last decade the need for new varieties resistant/tolerant to abiotic and biotic stresses has emerged. In order to reach this goal, the FEM pre-breeding activity targets the introgression of grapevine downy (GDM) and powdery (GPM) mildew resistance/tolerance into *vinifera* background, to be coupled with grape quality characteristics in the next future. In a woody species - as grapevine - the achievements of this long-term objective can be anticipated by means of continuous-flowering genotypes or *ad hoc* agronomical practices, such as fruiting cuttings.

The main objective is to create introgression lines in which the dilution of the *Vitis* spp. in the *V. vinifera* genome occurs with a selective and focused method based on the molecular detection of specific chromosome arms. With this aim, we focused on the genetic (190 SSRs) and phenotypic (GDM and/or GPM resistance) characterization of about 300 *Vitis* hybrids in order to identify selection signatures. Based on SSR profiles, the historical pedigree information has been checked and the trueness-to-type validated for several of the studied hybrids. This allowed to perform the Identity By Descent analysis, tracing the allelic flow through the successive generations. In particular, we exploited the information derived from the genome sequencing related to the presence of resistance gene analogue clusters along the chromosomes 5, 7, 9, 12, 13, 15 and 18.

The final result of this study will be the release of molecular markers valuable for grapevine Marker-Assisted Breeding, upon their validation in *ad hoc* segregating populations.

Genomics in azalea: defence against broad mite (*Polyphagotarsonemus latus*) infection as a case study

Ellen De Keyser [1], Gil Luypaert[1], Tom Ruttink [1], Johan Van Huylenbroeck[1] and Jan De Riek[1]

[1] *Plant Sciences Unit, ILVO, Belgium*

ellen.dekeyser@ilvo.vlaanderen.be

Plant material of different tissues (flowers, leaves and buds) from *R. simsii* genotypes was harvested after specific treatments. This resulted in 16 tissue samples for comprehensive transcriptome assembly. Six of these samples were related to plant defence: leaf samples of a mock treatment were compared to foliar application of methyl jasmonate (MeJa) or *P. latus* infestation on both a sensitive ('Nordlicht') and a mite tolerant ('Elien') genotype. The other samples were related to flower colour and branching. Poly-A selected RNA seq libraries were prepared and sequencing was performed using Illumina HiSeq PE-100. In total, 711M reads were used for de novo assembly according to the Orthology Guided Assembly routine described by Ruttink et al. (2013) using the *A. thaliana* proteome as a reference. The azalea transcriptome contains a total of 32367 transcript fragments representing the orthologs of 14860 unique *A. thaliana* genes; 8012 of these coding sequences presumably represent full-length proteins. For differential gene expression (DEG) analysis, reads were mapped to the *R. simsii* transcriptome using the RNA-seq analysis utility of CLCbio GW. In 'Nordlicht', more than 1400 genes were differentially expressed in response to either MeJa treatment or *P. latus* infestation; both treatments shared over 500 DEGs when compared to the mock treatment. For the mite tolerant genotype 'Elien' more than 2400 DEGs were reported upon mite infestation, but only 1342 DEGs were reported as a result of MeJa treatment. Compared to the mock, both treatments had 851 DEGs in common. Gene Ontology (GO) enrichment was analysed using the BINGO plugin for Cytoscape (version 3.2.1). Jasmonic acid (JA) involvement in the response to the treatment was clear in 3 out of 4 mock-treatment comparisons. Only when comparing the mock to *P. latus* infestation in 'Elien', no GO terms indicating the direct involvement of JA were enriched. These results provided evidence for the involvement of the JA-pathway in response to *P. latus* infestation. On the other hand, also genes involved in the salicylic acid (SA) pathway were differentially expressed. Hence, the DEG results were validated in gene expression analysis using genes for the biosynthesis pathways of JA (*LOX*, *AOS*, *AOC*, *OPR3* and *JMT*) and SA (*ICS* and *PAL*) and the *PPO* gene involved in oxidative stress response. Multiple experiments using either MeJa treatment or *P. latus* infestation on 6 different genotypes were used for this purpose. The results of this validation in relation to the transcriptome analysis will be discussed.

Azalea adaptation to adverse pH conditions: evaluation of potential resources for breeding

Sonia Demasi[a], Matteo Caser[a], Takashi Handa[b], Nobuo Kobayashi[c], Stefania De Pascale[d] and Valentina Scariot[a]

[a] *Department of Agricultural, Forest and Food Sciences, University of Torino, Italy*
[b] *School of Agriculture, Meiji University, Japan*
[c] *Faculty of Life and Environmental Science, Shimane University, Japan*
[d] *Department of Agricultural Sciences, University of Naples Federico II, Italy*

sonia.demasi@unito.it

Evergreen azaleas are world-wide sold pot ornamental plants belonging to the heather family (genus *Rhododendron*, section Tsutsusi). These shrubs suffer from iron (Fe) deficiency when the growing medium pH is higher than *optimum* (4.5-6.0). The main symptoms are the appearance of chlorosis in younger leaves and stunted growth. However, within wild azalea populations, genotypes tolerant to alkaline pH (up to 8) can be found. With the purpose to study Fe deficiency tolerance in azalea and select useful genetic resources for breeding, we conducted three experiments. In the first, we screened 11 genotypes (*R.* 'Juko', *R. indicum* 'Kinsai', *R. macrosepalum* 'Hanaguruma', *R. obtusum* 'Kirin', *R. obtusum* 'Susogo-no-ito', *R.* × *mucronatum* 'Fujimanyo', *R.* × *mucronatum* 'Ryukyushibori', *R.* × *pulchrum* 'Oomurasaki', *R.* × *pulchrum* 'Sen-e-oomurasaki', *R. scabrum*, and *R. tosaense*) in hydroponic conditions. Fe deficiency was imposed for 21 days by the addition of sodium hydrogen carbonate to the growing media. *R.* 'Juko', *R. scabrum*, *R. macrosepalum* 'Hanaguruma', *R.* × *pulchrum* 'Oomurasaki', and *R.* × *pulchrum* 'Sen-e-oomurasaki' showed lower foliar damages and mortality rates and were suggested as tolerant resources. In the second experiment, we compared the root Ferric Chelate Reductase (FCR) activity of the putative tolerant *R.* × *pulchrum* 'Sen-e-oomurasaki' and sensitive *R. obtusum* 'Kirin', maintaining the same experimental conditions of the first trial for 10 days. *R.* × *pulchrum* 'Sen-e-oomurasaki' showed an increased FCR activity under high pH, showing the typical behaviour of tolerant plants. Lastly, in the third experiment, we evaluated five azalea genotypes (*R. indicum* 'Shinsen', *R. indicum* 'Oosakazuki', *R. obtusum* 'Kirin', *R.* × *pulchrum* 'Sen-e-oomurasaki', *R. ripense*) under common cultivation practices. Cultivation occurred in a greenhouse for 10 weeks in pots filled with a peat: coconut fibre mix (1:1). Stress conditions were induced by direct Fe deficiency (through the addition of $CaCO_3$), indirect Fe deficiency (through simultaneous application of $CaCO_3$ and Fe fertilisation), and Fe absence. According to biomass production, morphological characteristics (canopy average diameter, plant height, leaf number) and physiological parameters (mineral elements, abscissic acid and chlorophyll content), multiple cross-talk signals were stimulated under adverse pH and azalea responses were genotype dependent. Quite surprisingly, *R. obtusum* 'Kirin' resulted moderately tolerant to alkalinity, while *R.* × *pulchrum* 'Sen-e-oomurasaki' suffered under direct Fe deficiency. *R. indicum* 'Shinsen' was extremely sensitive, with the highest Fe needs, while *R. indicum* 'Oosakazuki' and *R. ripense* were the most tolerant. Differences between hydroponics and pot cultivation responses highlighted the importance of long-term trials under common cultivation practices to evaluate adaptability to adverse pH conditions in evergreen azalea.

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Digital phenotyping for postharvest performance and development of a high-throughput genotyping platform for hexaploid chrysanthemum

Geert van Geest[abc], Uulke van Meeteren[a], Aike Post[b] and Paul Arens[c]

[a]*Horticulture & Product Physiology, Wageningen University, The Netherlands*
[b]*Deliflor Chrysanten B.V., The Netherlands*
[c]*Wageningen UR Plant Breeding, Wageningen University and Research Centre, The Netherlands*

geert.vangeest@wur.nl

In order to locate loci affecting postharvest performance in hexaploid chrysanthemum, we set up a phenotyping and genotyping platform. Sensitivity to disk floret degreening explains variation in post-harvest performance after long storage (van Geest et al., 2016). We selected two genotypes sensitive and insensitive to disk floret degreening. Degreening could be prevented by feeding the capitula with 50 mM sucrose (Figure 1), and it is therefore likely related to carbohydrate starvation. The two selected genotypes were crossed and this resulted in a progeny of 411 genotypes. The progeny was phenotyped by colour measurements of disk florets using a camera system and digital imaging. In order to genotype the population, we identified single nucleotide polymorphisms (SNP) by sequencing the transcriptome of the parents and 11 other cultivars. There is no sequenced genome of chrysanthemum available. Therefore, we assembled the transcriptome de novo. Because of the heterozygous and polyploid (2n=6x) nature of the crop we ended up with a highly redundant transcriptome of 227,213 transcripts. After read mapping and SNP calling, we used custom-made SNP filtering steps to reduce the amount of false-positives and identify false-duplicates, which resulted in 196,178 selectable SNPs. Preliminary genotyping with a selection of 97 SNP markers showed that different filtering pipelines resulted in 20 to 68% properly segregating SNPs that can be used for linkage mapping. We are currently developing a genotyping array to genotype our population with >100,000 SNPs. We will combine this genotypic data with the phenotypic data to identify quantitative trait loci for disk floret degreening.

Figure 1. QR code coding for a web-link containing a time-lapse video: https://youtu.be/-NgTSWUvI4Y. For this video, capitula were photographed every 15 minutes during 14 days. The ray florets were removed and the stem ends were placed in 25 mg/L sodium dichloroisocyanurate (DICA; first two rows) or 25 mg/L DICA + 50 mM sucrose (third and fourth row). The first and third row represent the selected genotype that is insensitive to disk floret degreening, whereas the second and fourth row represent the sensitive genotype. Scan the code with a mobile device for quick access to the video.

Van Geest, G., Choi, Y.H., Arens, P., Post, A., Liu, Y., van Meeteren, U., 2016. Genotypic differences in metabolomic changes during storage induced-degreening of chrysanthemum disk florets. Postharvest Biol. Technol. 115, 48–59.

Honeybush breeding: revealing the mysteries of this South African indigenous crop

Cecilia Bester[a,b], Mlamuli M. Motsa[a,b], Retha M. Slabbert[b] and Kenneth R. Tobutt

[a] *Cultivar Development and Horticulture, ARC Infruitec Nietvoorbij, South Africa*
[b] *Horticulture Department, Tshwane University of Technology, South Africa*

besterc@arc.agric.za

Honeybush (*Cyclopia* spp.), a traditional South African herbal tea unique to the Western and Southern Cape regions, has become popular world-wide. Its popularity is due to its caffeine-free and comparatively low tannin status, combined with potential health-promoting properties especially antioxidant activity. However, the demand for this herbal tea put pressure on wild populations as a source of seed and of fresh biomass for processing. More than 70% of the tea is still harvested from the wild and, with only ±150 hectares under commercial production, the need for improved and sustainable production was recognised. To address this need the ARC initiated an improvement programme for honeybush in the late 1990s. The aim of this programme is to improve the commercial characteristics (growth, yield and chemical properties) of the different species through intra-species crosses, selecting promising individuals from the crosses and evaluating the selections in different climatic regions for adaptability. Continuous improvement of the different species will ensure that new and improved material becomes available on a regular basis for commercial purposes. However, with very little basic genetic information available, e.g. chromosome number, ploidy level and incompatibility, breeding soon became a challenge especially with controlled crosses. Propagation was another challenge and to develop propagation protocols was of upmost importance. Several complementary research projects have helped to discover the secrets of this new exciting crop. Today chromosome numbers and ploidy level of three of the important commercial species have been confirmed with cytogenetic studies as well as with flow cytometry analysis. Flow cytometry can now be used for quick determination of ploidy levels of new selections. The use of SSR markers is currently under investigation for fingerprinting selections and for population genetics studies. Early indications of broad sense heritability are about 0.4 for yield and, simply through better selection and using an open pollinated polycross strategy, yield was increased by at least 3 tonnes (35%) per hectare. Propagation protocols, for seeds and cuttings, have been developed and are used for commercial deployment of honeybush. The breeding programme is still in its initial phase, but in recent years valuable information and results have been obtained for this formerly unknown agricultural crop. This information will form the basis for the breeders to continue the improvement of this new crop and to ensure that the emerging industry becomes sustainable.

Irrigation practices differently affect the emission of biogenic volatile organic compounds in *Helichrysum petiolare* and *Salvia sinaloensis*

Matteo Caser[a], Francesca D'Angiolillo[b], Barbara Ruffoni[c], Luisa Pistelli[d], Laura Pistelli[b], Valentina Scariot[a]

[a] *Department of Agricultural, Forest and Food Sciences, University of Torino, Grugliasco (TO), Italy*
[b] *Department of Agriculture, Food, and Environment, University of Pisa, Pisa, Italy*
[c] *CREA-FSO, Ornamental Species Research Unit, Sanremo (IM), Italy*
[d] *Department of Pharmacy, University of Pisa, Pisa, Italy*

matteo.caser@unito.it

Biogenic volatile organic compounds (BVOCs) are abundant chemicals emitted by organisms in all ecosystems. The most common BVOCs belong to four major classes: phenylpropanoids/benzenoids, terpenoids, amino acid derivates, and fatty acid derivates. Nowadays more than 1700 different BVOCs have been identified in at least 90 different plant families. They are mainly known to play roles in plant signal transduction and to mediate interactions between plants and other organisms. Their emission is differentially regulated and strongly dependent on response to abiotic and biotic stimuli, even if mechanisms are not well defined yet. In particular, the emission of BVOCs in response to different cultivation practices is still a debated question.

This study analysed and compared the influence of irrigation on the emission of BVOCs in two medicinal and aromatic plants (MAPs): *Helichrysum petiolare* and *Salvia sinaloensis*. Three water regimes were applied: 100% of container capacity (CC, full irrigation), 50% CC (moderate water stress) and 0% CC (no irrigation, severe water stress).

The two species responded differently to irrigation regimes. Under stress conditions, the total amount of BVOCs was not affected in *H. petiolare* while it increased in *S. sinaloensis*. Conversely, BVOCs quality changed in both genotypes. Interestingly, monoterpene hydrocarbons increased in moderate stressed plants (~+27%) and oxygenated monoterpenes increased with stress severity (~+37% and ~+123% in moderated and not irrigated plants, respectively). Thus, drought stress does not inhibit *per se* the biosynthesis of this class of compounds. Besides increasing knowledge about the ecophysiological control of constitutive or induced BVOCs, these findings could be exploited by the industry to improve MAP production quality and save water.

Parallel oral presentations: Legumes

In situ conservation of bean germplasm from Northwestern Argentina

Mariana J. Ferreyra[a], María del Carmen Menéndez Sevillano[a], Luis R. Ibarra[a] and **Antonio M. De Ron**[b]

[a] *National Agricultural Technology Institute (INTA)-Active Bank of Northwestern Argentina (BANOA), EEA Salta, Argentina*
[b] *Misión Biológica de Galicia (MBG)-Biology of Agrosystems, National Spanish Research Council (CSIC), Pontevedra, Spain*

amderon@mbg.csic.es

Genetic resources for agricultural development and future food security are constantly threatened therefore its conservation is imperative. *In situ* conservation ensures continuity of dynamic processes in which species develop under environmental changes and co-evolve with biotic and abiotic factors, therefore, the processes of evolution and adaptation are maintained and the generation of new genotypes is guaranteed.

Utilization and farm conservation throughout generations is recognized as a type of *in situ* conservation through which farmers and local communities conserve the genetic diversity in agro-ecosystems where they have traditionally maintained their crops and ancestral knowledge associated with the resource. *In situ* conservation of wild species is an alternative of conservation that allows keeping populations in their natural environments, and set the strategies for maintaining populations.

Since 2006 the National Agricultural Technology Institute (INTA) of Argentina has begun the development of *in situ* conservation projects. The Active Bank of Northwestern Argentina (BANOA) has coordinated the *in situ* conservation of primitive varieties and wild populations of common bean (*Phaseolus vulgaris* L.).

The BANOA has an important collection of native bean germplasm that has been collected from northwestern Argentina over 20 years, including 500 landraces and 222 wild accessions. Landraces have been the basis for reinsertion into farmers' fields. Together with extensionist technicians was made a survey of small farmers in the Andean region who were interested in receiving local germplasm. They were given visually selected populations and they were accompanied in monitoring their crops. Workshops to train them in preserving their own seeds with good quality and where the exchange of knowledge took place were also conducted.

For wild bean, presence and genetic diversity of populations was evaluated along its distribution range in northwestern Argentina both in protected and unprotected areas. It was found that most of the populations were found in places modified by human activities. According to variability studies made on the basis of morphological characters, the most variable populations corresponded to the Ecoregion Forest of Yungas, so this would be the most appropriate to establish the genetic reserve areas for bean wild populations.

Unlocking and enhancing nature's diversity to benefit breeding strategies for diverse end uses in pea

Claire Domoney[a], Tracey Rayner[a], Carol Moreau[a], Mike Ambrose[a], Alfonso Clemente[b] and Peter G. Isaac[c]

[a] *John Innes Centre, Norwich Research Park, UK*
[b] *Estación Experimental del Zaidín, Granada, Spain*
[c] *IDna Genetics Ltd, Norwich Research Park, Norwich, UK*

claire.domoney@jic.ac.uk

Considerable allelic variation exists outside standard breeding genetic pools in *Pisum sativum* L. (pea). Unlocking this variation provides resources which not only further our understanding of the genetic control of economically important traits, but which may be exploited ultimately within breeding programmes. Induced mutant and natural germplasm populations are proving to be equally valuable resources for isolating allelic variants of candidate genes involved in the control of seed quality in pea, including visual and compositional traits, and for underpinning the development of molecular markers to facilitate the introgression of novel alleles from exotic gene pools.

A pea germplasm resource (Jing et al. 2010) and mutagenized populations (Dalmais et al. 2008; Domoney et al. 2013) have been screened by a number of high-throughput methods. Selected variant lines are being backcrossed to a recurrent parent line to provide near-isogenic lines suitable for field and industrial assessments. Variants for seed composition differ primarily in the concentrations of total starch, resistant starch, sugars, total protein and anti-nutritional proteins. As an example of the last group, whereas induced mutant populations yielded lines in which trypsin/chymotrypsin inhibitor activity was reduced by up to 60%, a high-throughput germplasm screen identified an extremely rare null allele, in a *Pisum elatius* accession, where a short deletion exists in both of the closely linked genes which encode the major seed inhibitors (Clemente et al. 2015). Combining this variant with mutations affecting lectin and pea albumin 2 genes (Domoney et al. 2013; Vigeolas et al. 2008), is providing opportunities for considerable gain in nutritional quality in pea seeds. Additional losses of seed protein genes are providing genetic stocks to underpin our analysis of the relationship between protein quantity and overall seed yield. Similarly, selected mutations that affect the accumulation of starch and sucrose-derived oligosaccharides show how quality may be improved without detriment to plant health and overall yield.

Visual traits are important to breeding programmes for food crops, as they influence economic value. Loss of colour from seed products may be avoided by mutations affecting regulation of the chlorophyll degradation pathway, avoiding perturbations in chlorophyll turnover which diminish yield under stress conditions (Bell et al. 2015).

Evaluation of new breeding lines of white lupin with improved resistance to Anthracnose

Irene Jacob[a], Manuel Deyerler[b], Ulf Feuerstein[c], Markus Heinz[b], Dörte Schweneker[c] and Peer Urbatzka[a]

[a] *Institute for Organic Farming, Soil and Resource Management, Bavarian State Research Center for Agriculture, Germany*
[b] *Saatzucht, Landwirtschaftliche Lehranstalten Triesdorf, Germany*
[c] *Deutsche Saatveredelung AG, Germany*

Irene.Jacob@LfL.Bayern.de

Since the mid-1990s, the cultivation of white lupin (*Lupinus albus* L.) has rapidly decreased in Germany and other countries in Central Europe because of the occurrence of *Colletotrichum lupini*, the causal agent of the fungal disease anthracnose, and the lack of varieties with a sufficient resistance towards this disease. To re-establish the relevance of cultivating white lupins, it is vital to develop new varieties with improved resistance.
In the study presented here, breeding lines generated by the breeding station of the Landwirtschaftliche Lehranstalten Triesdorf were evaluated from 2012 to 2014 on a total of five testing sites in Germany and one in the Netherlands. In each year, at least on one site a high disease pressure with good differentiation built up from natural infestations, so that further selection for resistance was possible in all three years. The breeding lines showed improved performance of resistance towards *C. lupini* on all testing sites compared to the reference varieties. A differentiation among the breeding material could not only be observed for resistance, but also for other agronomic traits such as plant length and alkaloid content. Improved resistance has a favourable effect on grain yield, which is best demonstrated in years with high disease pressure, and yield stability. The best lines will be intended for registration as variety. After the successful registration process new types of white lupins will be available for cultivation.

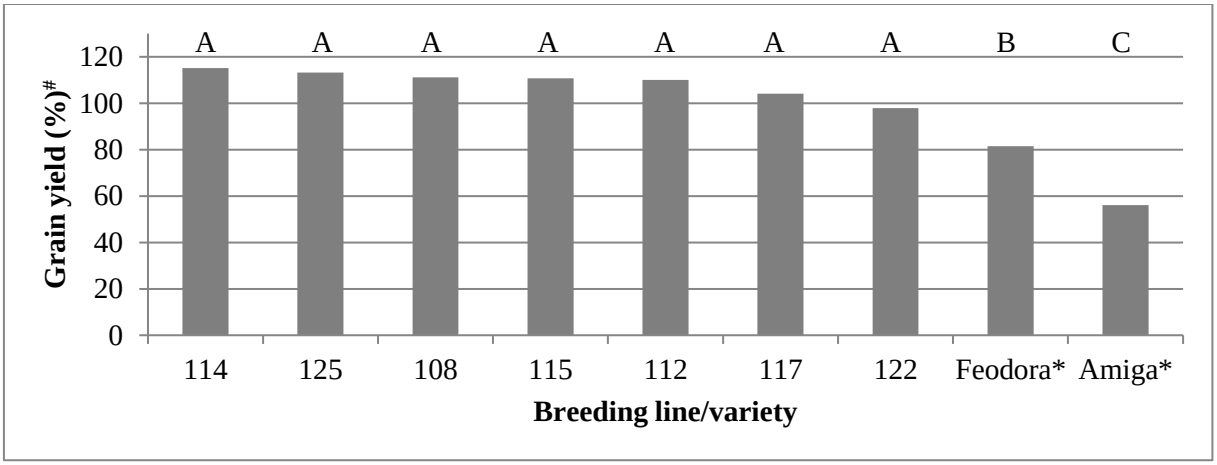

Figure: Grain yield (%) of breeding lines and varieties of white lupin. #) adjusted means from three years, eleven environments, 100 % = 26.0 dt ha^{-1}. *) Feodora nine environments, sown in Hohenkammer 2012 14 days later; Amiga ten environments. Different letters show significant differences ($p < 0.05$, Student-Newman-Keuls test).

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Resistance to Powdery mildew in pea germplasm

Diego Rubiales, María J. González-Bernal and Sara Fondevilla

Institute for sustainable Agriculture, CSIC, Córdoba, Spain

diego.rubiales@ ias.csic.es

Pea powdery mildew is an air-borne, worldwide-distributed disease caused mainly by *Erysiphe pisi* but can also be caused by other species like *E. trifolii* and *E. baeumleri*. Pea breeding is based largely on the use of *er1* resistance gene, with little use of the second available gene *er2*. Even when *er1* is regarded to provide a more durable type of resistance and today we know that in fact it is a *mlo* type; it is safer to have a broader battery of genes available to pre-event/delay resistance breakdown. For instance, It has been recently found that *er1* is overcome by *E. trifolii*.

In order to extend the availability of sources of resistance, we started by screening a germplasm collection resulting in the identification of quantitative and qualitative resistance to *E. pisi*. Particularly relevant was *Pisum fulvum* germplasm where we identified a new gene for resistance to *E. pisi* that we called *Er3*. Interspecific hybridization between *P. fulvum* and *P. sativum* is possible although hampered by low fertility and many other detrimental traits. We have been able to introduce *Er3* into *P. sativum* background by backcrossing and selection for resistance and fertility, resulting in the release of a resistant pea cultivar ("Eritreo") containing this gene. We have identified SCAR markers linked to this gene that will facilitate the early selection of individuals carrying the gene in breeding programs. The mode of action at the cellular level of the three resistance genes has been characterized, and genes and proteins involved in the resistance have been identified.

At present we are approaching a deeper understanding of the molecular and biochemical mechanisms of response to *E. pisi* in pea and looking also for resistance to *E. trifolii*, and developing the tools and knowledge needed for the introgression of the resistance into pea cultivars.

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Salt tolerance screening of the AVRDC mungbean (*Vigna radiata*) collection

Ching-hunan Chang[a], Chun-Yu Shen[a], Wuu-Yang Chen[a], Jo-yi Yen[a], Ramakrishnan Nair[b] and **Svein O. Solberg**[a]

[a] *AVRDC – The World Vegetable Center, Headquarters, Taiwan*
[b] *AVRDC – The World Vegetable Center South Asia, India*

svein.solberg@worldveg.org

High salinity soil is a major challenge for food crop cultivation in dry and warm climates where irrigation is needed but water is in shortage. In these locations mungbean is an important pulse crop for cultivation in rotation after the main grain harvest. Integrating improved mungbean varieties can be part of a strategy for increasing smallholder farmer income and ensuring more sustainable production systems. At AVRDC – The World Vegetable Center, a floating hydroponic screening system for salt stress tolerance has been developed. Plants are grown at 30 ºC on foam blocks fixed in Styrofoam board floating on a nutrient solution containing different concentrations of salts. To establish a salt tolerance screening protocol for mungbean, solutions of 0 mM, 25 mM, 50 mM, and 75 mM NaCl or $MgSO_4$ were tested. NaCl delayed germination but most plants survived, at least at the lower doses. Almost all plants failed to grow well with $MgSO_4$, especially at the higher doses, but even 25 mM caused a severe reduction in plant growth. A protocol based on 50 mM NaCl was used for further screening. The AVRDC mungbean mini-core collection, 296 accessions, was screened. The mini-core is a subset of the entire AVRDC collection of more than 3000 mungbean accessions developed to capture the diversity in the whole collection. At the germination stage, two accessions showed no reduction in germination and a further eight accessions showed more than 90% germination under salt stress conditions. At the seedling stage, seven highly tolerant accessions were identified, but these were not the same as those accessions showing the highest tolerance at germination, indicating that different mechanisms are involved in the two stages. From a combined screening using seedlings and plants up to two months old, 60 accessions were selected as promising and are undergoing further examination. The best lines will be shared with breeders and be used to develop extra-early maturing, drought- salt- and heat-tolerant varieties for production in mid-summer to late autumn for the dryland systems of South and Central Asia. The mini-core collection is also being examined under field conditions in targeted areas in Pakistan and Uzbekistan.

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Grain yield potential and stability of large-seeded vegetable-type soybean genotypes

Rouxléne van der Merwe[a], Armand Smit[b], Michiel Smit[b] and Maryke T. Labuschagne[a]

[a]*Department of Plant Sciences, University of the Free State, Bloemfontein, South Africa*
[b]*Edamame Development Program, Hillcrest, KwaZulu-Natal, South Africa*

vandermerwer@ufs.ac.za

Vegetable-type soybean has recently been introduced to South Africa as a supplementary high quality protein food source that is eaten as a green vegetable. The crop serve as a new nutritional crop for small-scale community farming, while 81 emerging farmers currently grow vegetable-type soybean under contract. However, lack of adapted cultivars is one of the major constraints in commercial seed production of this crop. The aims of this study were to evaluate and identify genotypes for potential production in South Africa, to identify suitable parents that could be used in the breeding programme and to identify the most stable location for the selection programme. A two-year study (2013/2014-2014/2015) was performed to evaluate 16 non-genetically modified cultivars, across five locations for various traits including days to flowering and maturity, plant height, pod height, number of nodes per plant, number of branches per plant, 100-seed weight and seed yield. Combined analysis of variance indicated highly significant ($P<0.01$) variation among genotypes for all traits analysed. Genotypes showed large variation in days to flowering (44-75 days) and maturity (100-167 days). Grain yield ranged from 1.35-2.34 t ha^{-1} with 100-seed weight ranging from 16-40 g. Highly significant genotype x environment interaction effects were observed for grain yield and as a result multivariate analysis (AMMI) was done. AMMI biplots revealed that Pinetown showed the highest yield potential, but was the most unstable location. Winterton was the most stable location for grain yield, but showed below average yield potential. Of the top three yielding genotypes AGS354, AGS418 and AGS352 only AGS418 showed acceptable adaptability across locations and seasons. From this study, potential genotypes have been identified that could be utilised as a source of traits in the vegetable-type soybean breeding programme. In future fresh pod yield and quality will be evaluated in order to make selections for the fresh market. Additional locations need to be included and evaluated in the variety trials in order to find a more suitable location for the selection programme.

Polyamines in legumes: Components with a possibly strong health potential and their respective breeding options

Johann Vollmann[a], Tatsuya Sagara[a], and Helmut K. Mayer[b]

[a] *Plant Breeding Division / Department of Crop Sciences, University of Natural Resources and Life Sciences Vienna (BOKU), Austria*
[b] *Institute of Food Science / Department of Food Science and Technology, University of Natural Resources and Life Sciences Vienna (BOKU), Austria*

johann.vollmann@boku.ac.at

Polyamines such as putrescine, spermidine or cadaverine have numerous different functions in plants as well as in food products and in human nutrition. Recent findings have identified spermidine and possibly some other polyamines as major health protecting components with strong anti-aging effects as revealed in cellular and animal models. Food legumes such as soybean, pea, cowpea or adzuki bean and a number of legume-derived food products have been found to be the richest sources of polyamines as compared to cereals, vegetables or fruits which might partly explain the health benefits associated with legume consumption. Therefore, for the first time genetic variation in polyamines and the respective plant breeding options of selecting for these components have been evaluated in the present case study for soybean. A set of early maturity soybean genotypes differing in seed protein content was grown in a replicated field experiment at Gross Enzersdorf near Vienna, Austria for three seasons. Polyamine concentrations were determined from seed samples after harvest using an ultra-high performance liquid chromatography (UHPLC) approach. The results confirm earlier reports on soybean polyamine levels: Spermidine concentration was in the range of 167-291 mg/kg dry seed, whereas putrescine and cadaverine were between 3 and 29, and spermine between 31 and 179 mg/kg, respectively. Statistically significant genetic as well as environmental variation was found for all polyamines analysed. Concentrations of putrescine, spermidine and spermine were highly correlated to each other which is due to their common biosynthesis pathway. Seed protein content was negatively correlated to cadaverine but not correlated to any other soybean polyamine. Estimates of heritability for polyamines were medium to high and comparable to other seed quality traits suggesting that polyamine concentrations could be selected for in food-grade soybean breeding. However, a major limiting factor in breeding for spermidine level and other seed quality components of food grade soybean is the high cost of chemical analysis permitting the screening of large numbers of breeding lines. With respect to spermidine, a preliminary near-infrared reflectance spectroscopy (NIRS) calibration with limited accuracy (validation R^2 = 0.60) has been developed so far for rapid screening of breeding materials. Applying that calibration in a number of larger soybean experiments, reproducible predictions of spermidine concentrations in breeding lines have been obtained suggesting that a rough classification of genotypes is possible within selection experiments. Thus, plant breeding could contribute to the development of food grade soybeans with an additional health benefit.

Parallel flash and poster presentations: Wheat

Nicotianamine synthase genes as a valuable genetic resource for improving bread wheat growth and nutrition

Julien Bonneau[a], Jesse Beasley[a], Ute Baumann[b], Yuan Li[b] and Alexander Johnson[a]

[a] *School of BioSciences, The University of Melbourne, Victoria, Australia*
[b] *Australian Centre for Plant Functional Genomics, The University of Adelaide, Australia*

julien.bonneau@unimelb.edu.au

Nicotianamine synthase (NAS) genes encode enzymes that synthesize nicotianamine (NA); a non-protein amino acid that functions as an important chelator of ferrous iron (Fe^{2+}) and other divalent metal cations in plants. Nicotianamine is also the biosynthetic precursor to mugineic acid family phytosiderophores that graminaceous plant species, such as bread wheat (*Triticum aestivum* L.), secrete into the rhizosphere to absorb ferric iron (Fe^{3+}) through Strategy II iron uptake. This project aimed to identify and characterize the NAS genes of bread wheat to provide a novel genetic resource for plant breeders working to improve bread wheat growth under iron excess/limiting conditions as well as grain nutrition. We used the 10 NAS genes of barley (*Hordeum vulgare* L.) in BLAST searches of the International Wheat Genome Sequencing Consortium survey sequences and identified 21 single-exon NAS genes on multiple chromosomes and all three sub-genomes of bread wheat. Phylogenetic analyses of the bread wheat NAS genes with those of rice (*Oryza sativa* L.), maize (*Zea mays* L.) and barley showed that the wheat NAS genes separate into two distinct clades, with 18 NAS genes belonging to clade I and 3 NAS genes belonging to clade II. The majority of NAS genes from both clades were highly expressed during grain development, germination and seedling growth demonstrating enhanced need for metal transport and homeostasis at these stages. Furthermore, expression of 14 of the clade I NAS genes was significantly up-regulated in the roots of wheat plants grown under conditions of iron deficiency. Identification and characterization of the large NAS gene family in bread wheat opens new doors for breeding and biotechnological strategies aimed at improving the growth and nutritional value of bread wheat.

The manifestation and phytohormone response of leaf pubescence genes in bread wheat

Alexey Doroshkov, Alexander Simonov, Dmitry Afonnikov and Tatyana Pshenichnikova

Institute of Cytology and Genetics Siberian Branch Russian Academy of Sciences, Russia

Email: ad@bionet.nsc.ru

The leaves of many angiosperm species develop trichomes. This trait is known to make a significant contribution to the protection from pests and adaptation to environmental factors in bread wheat.

However the genetic basis of wheat trichome formation is poorly understood although a wide variation was found among *Triticeae* species with different ploidy level. Currently Catalogue of Gene Symbols for wheat contains only two loci associated with this trait: the gene *Hl1* in 4B chromosome and the gene $Hl2^{aesp}$ in 7B chromosome. Molecular function and regulation of these genes are currently not known.

The present research sought to establish the individual and joint effect on trichome patterning and growth of each of three wheat leaf pubescence genes (*Hl1*, $Hl2^{aesp}$ and new one - *Hl3*) under normal conditions and phytohormone treatment.

Various lines carrying *Hl1*, *Hl3* and $Hl2^{aesp}$ and specially created nearly isogenic lines were used to quantitatively compare leaf pubescence using a modern high throughput phenotyping method (wheatdb.org/lhdetect2). This method allows us to obtain rapidly quantitative characteristics of leaf pubescence (length of individual trichomes and their number) among many plants.

Studied genes differed in their effect on trichome formation. *Hl1* and *Hl3* more affected trichome initiation and growth, while $Hl2^{aesp}$ modified mostly trichome length. Their action was independent to a large extent. A model of the action and interaction of *Hl1*, *Hl3* and $Hl2^{aesp}$ has been proposed to explain the genetic basis of trichome length and number.

The effects of phytohormones on trichome cell growth and initiation while *Hl1*, *Hl3* and $Hl2^{aesp}$ genes manifestation were explored. The effects of auxin (IAA), gibberellic acid (GA), cytokinins (6-BAP, Kinetin), metyl jasmonate (MeJa), ethylene (ACC) have been investigated and described. Our data revealed a key role of GA and cytokinin signaling pathways in *Hl1* and *Hl2* gene manifestation. At the same time this genes differs in a character of response to hormone action. This suggests a different position *Hl1*, *Hl3* and $Hl2^{aesp}$ genes in the network of trichome formation control.

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Lr22a gene is effective and does not alter other disease resistances, yield or bread making quality

Odile Moullet[a], **Dario Fossati[a]**, Cécile Brabant[a], Andrea Marti[a], Fabio Mascher[a], Simon Krattinger[b] and Arnold Schori[a]

[a] *Institute for Plant Production Sciences/Plant Breeding and Genetic Resources, Agroscope, Switzerland*
[b] *Department of Plant and Microbial Biology, University of Zurich, Switzerland*

odile.moullet@agroscope.admin.ch

The efficacy and unintended effects of new resistance genes must be examined before utilising the latter in a breeding programme. In the case of wheat, it is necessary to verify that the resistance gene is still effective, but has no negative effect on other disease resistances, yield, or baking quality.
The *Lr22a* gene confers resistance to leaf rust at the adult stage (adult-plant resistance), and microsatellite markers linked to it have been identified (Hiebert et al., 2007). To date, this gene is relatively rarely used, and is still effective under Swiss conditions.
Lr22a was introgressed from the Canadian spring wheat cultivar AC Minto by 6 backcrosses (BCs) into the susceptible Swiss spring wheat cultivars CH Campala and CH Rubli. The presence of *Lr22a* was examined at each BC step using the microsatellite markers wmc503 and gwm261. The homogeneity of the resulting two backcross lines (BC lines) CH Campala-6BC and CH Rubli-6BC with their recipient cultivars was verified using a 15K SNP Array.
The BC lines CH Campala-6BC and CH Rubli-6BC were compared with their original cultivar in multi-location yield trials for four (2012 to 2015) and two (2012 to 2013) years respectively. Simultaneously, resistance to stripe rust, powdery mildew, leaf and glume blotch (*Phaeosphaeria nodorum*), septoria leaf blotch (*Mycosphaerella graminicola*) and fusarium head blight (*Fusarium graminearum*) were tested with artificial infections. Protein content was tested and Zeleny sedimentation index was determined in each location. The seed harvested from all locations was used for dough- and bread-making quality tests (farinograph, extensograph and Rapid-Mix Test).
The original cultivars (CH Campala and CH Rubli) and their essentially derived lines containing *Lr22a* (BC lines) were very similar in terms of heading time, plant height and morphological traits. Overall, the BC lines showed significantly improved resistance to leaf rust in all trials. In years (particularly 2012) and at locations with strong leaf rust pressure, we measured a significant yield reduction in the original varieties compared with the improved backcross lines. Apart from the appearance of a few stem rust symptoms on the backcross line CH Campala-6BC in one trial, resistance to all other diseases was not affected. Possible differences in stem rust susceptibility have yet to be investigated.
No significant (or very small) differences were observed for protein content, Zeleny index, and rheological or baking parameters.
In conclusion, the *Lr22a* gene has proved effective against leaf rust, with no associated negative effects. Nevertheless, we recommend associating this gene with one or more effective leaf rust resistance genes in order to ensure its durability.

Hiebert, C., et al. (2007). 'Microsatellite mapping of adult-plant leaf rust resistance gene *Lr22a* in wheat', TAG Theoretical and Applied Genetics **115(6)**: 877-884.

A large-scale association mapping analysis of wheat resistance to multiple fungal pathogens across three years in multiple locations in NW Europe

Keith Gardner[a], Pauline Bansept-Basler[ad], Ian Mackay[a], James Cockram[a], "Wagtail" Consortium[b] and Donal O'Sullivan[c]

[a] *Genetics and Breeding, NIAB, UK*
[b] *A group of 8 wheat breeding companies, NIAB and John Innes Centre, UK/NW Europe*
[c] *School of Agriculture, Policy and Development, Reading University, UK*
[d] *Current address: Syngenta, France*

keith.gardner@niab.com

Evaluation of resistance to multiple diseases over multiple sites and years using the same breeding pool is a powerful method for identification of resistance loci and subsequent improvement of breeding programs. To identify new sources of disease resistance in NW European wheat (*Triticum aestivum* L.), we performed a genome-wide association study (GWAS) using a collection of 480 UK and related NW European wheat varieties. This association mapping panel was genotyped with the Illumina Infinium iSelect 90K SNP array, which generated >26000 SNPs usable for GWAS. In a series of more than 30 field trials over 3 years in 12 locations across 5 countries, adult plants were evaluated for resistance to four of the most important fungal diseases of wheat in NW Europe: yellow rust (caused by *Puccinia striiformis* f. sp. *tritici*), brown rust (*Puccinia triticina*) septoria tritici blotch (*Mycosphaerella graminicola*) and powdery mildew (*Blumeria graminis*). Between 20 and 78 unique resistance loci per disease were identified using GWAS with a false discovery rate (fdr) threshold of 0.05. Of these, between 8 and 20 hits per disease were considered of potential use to plant breeders, as the resistant alleles were not already at high frequency in breeders' germplasm. Overall, the largest number of resistance hits was identified for yellow rust (YR), which also showed a distinctive temporal pattern: several major hits identified in the first year trials were absent in the third year trials, and vice versa. This pattern correlated with dramatic race changes in the pathogen population over the 3 year course of the field trials. However, several YR loci were also detected across all years, and are potentially the most valuable for providing durable adult plant resistance. For YR, a validation exercise was conducted, using 19 biparental populations selected from current breeders' crosses. Of 13 breeder-relevant hits for which suitable variation was available, 12 were successfully validated. In addition, we successfully validated a further 4 of 13 "marginal" breeder-relevant hits (fdr >0.05, unadjusted P value <0.0001). We then performed a cross-disease analysis to identify potential broad-spectrum resistance loci, as well as interactions between resistance-associated loci for the four different diseases, and the implications of these for plant breeding. Finally, we directly compared our results using the GWAS panel to results from field trials using our NIAB 8-parent wheat multiparent advanced generation inter cross (MAGIC) population, which was genotyped with the same array.

Winter wheat and climate change adaptability – finding functional markers in elite wheat germplasm

Sunčica Guberac, Sonja Petrović, Andrijana Rebekić and Sonja Marić

Faculty of Agriculture/Department of Plant Production, University of J.J. Strossmayer, Croatia

suncicag@pfos.hr

Wheat is dominant crop in temperate climate and its success depends on its adaptability and high yield as well as specific quality traits. Large natural variation of this species is enabled by its hexaploid genome structure. Adaptation of wheat genotypes to diverse environmental conditions is under strong influence of flowering time. The aim of this study was to detect presence of functional markers for plant height, response to photoperiod and vernalisation requirement among 70 elite wheat germplasm. Selected cultivars, originated from Croatia, Serbia, Italy Austria, Germany, France, Hungary, Russia, were evaluated for plant height and flowering date in field trial located in Eastern part of Croatia. DNA extraction from three phase leaves was carried out by CTAB method. Average plant height was 88.92 cm, ranged from 62 to 159.32 cm with CV of 19.23%. Days to heading ranged from 142 to 119 days with CV of 4.06%. Our results indicated that Rht-B1b, Rht8 (192bp), Ppd-D1a dominate with frequencies of 62%, 68%, and 72% respectively, followed by Rht-D1a, Rht-D1b and Ppd-D1b alleles. Seventeen cultivars with Rht-B1a and Rht-D1a had plant height ≥98 cm, while 25 cultivars carrying dwarf alleles had plant height from 62 to 96 cm. Among Croatian cultivars Vrn-A1a was detected in only one cultivar, Vrn-A1c in three while vrn-A1 dominated with 92%. Prevalence of vrn-B1 and vrn-D1 was recorded with 95% and 100%.

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A snapshot on wheat breeding for the Canadian Prairies

Santosh Kumar[a], Andrew Burt[a] and Jennifer Mitchell-Fetch[a]

[a]*Brandon Research and Development Centre, Agriculture and Agri-Food Canada, Canada*

Santosh.kumar@agr.gc.ca

Canada ranks sixth in the world for hard red spring wheat production, the vast majority of which is produced in western Canada. Most large producers of wheat (i.e. China, India) sustain their large populations on their domestic production of wheat. Canada is somewhat unique as approximately 75% of its total wheat production is exported. In 2014, Canada produced more than 29 million tonnes of wheat of which 24 million tonnes were exported contributing approximately $7.9 billion dollars to the Canadian economy. Spring hexaploid wheat accounts for 69% of the total wheat production followed by durum wheat (23%) and winter wheat (8%). Approximately 96% of the wheat is grown in the prairie provinces of Alberta, Saskatchewan and Manitoba. Due to warmer summers and higher rainfall, the eastern Prairies are home to some of the highest wheat grain yields in western Canada but also are hotspots for major wheat diseases such as rusts, Fusarium head blight (FHB), common bunt and loose smut as well as insects such as wheat blossom midge and wheat stem sawfly. Among the nine major Canadian wheat classes, Canada Western Red Spring (CWRS) has the highest acreage followed by Canada Western Amber Durum (CWAD). The CWRS class is characterized by its premium and stable quality attributes of high protein and strong gluten strength. Wheat breeding for CWRS class requires optimum disease resistance to priority diseases (FHB, leaf rust, stem rust, stripe rust, loose smut, leaf spot diseases and common bunt) as well as stringent screening for quality attributes (protein content, milling performance, gluten strength and baking quality). From initial cross to release of a finished cultivar the process takes ten to twelve years and each line is rigorously tested and thoroughly characterized with seven years of multi-location testing, representing about 40 environments (location/years). Superior lines are reviewed by a variety recommending committee comprising of agronomy, disease and quality sub-committees and the Central Food Inspection Agency administers the variety registration process and protection of plant breeder's rights. The poster presentation will elaborate on breeding techniques, major diseases and their sources of resistance and a breeding flowchart outlining key steps involved in wheat breeding for Canadian Prairies.

Resistance to Fusarium head blight of winter wheat lines derived from crosses between winter type cultivars and resistant spring wheat 'Sumai 3'

Tomasz Góral[a], Dorota Walentyn-Góral[a], Magdalena Radecka-Janusik[b], Paweł Czembor[b] and Halina Wiśniewska[c]

[a] *Department of Plant Pathology, Plant Breeding and Acclimatization Institute - National Research Institute, Radzików, Poland*
[b] *Department of Plant Breeding and Genetics, Plant Breeding and Acclimatization Institute - National Research Institute, Radzików, Poland*
[c] *Department of Genomics, Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland*

t.goral@ihar.edu.pl

Lines of winter wheat were obtained from crosses between Polish winter wheat cultivars ('Begra', 'Korweta' and 'Turnia') and resistant to Fusarium head blight spring wheat cultivar 'Sumai 3', which was a donor of highly effective FHB resistance gene – *Fhb1*. Lines were selected using pedigree method on the basis of their resistance to FHB (after *Fusarium* inoculation), resistance to other diseases and morphological characters. The best 52 lines of F_{10} generation were tested for FHB resistance in field experiments in two locations. Resistance of type I and II was tested under partially controlled conditions. Presence of *Fhb1* gene was screened using UMN10 marker closely linked to this gene. Resistant allele of UMN10 was detected in 56% (29) of lines which indicates that they carry *Fhb1* gene. Average FHB index was significantly lower for lines with *Fhb1*. The height of lines carrying *Fhb1* gene were significantly increased than ones without the gene. Grain yield per plot varied widely. The lowest yield exhibited tallest lines with *Fhb1*. Grain yield was strongly affected by yellow rust infection and all low-yielding lines were susceptible to this disease. Majority of lines (33) were resistant to yellow rust. Using multivariable analysis we were able to identify 13 lines combining favorable features: high resistance to FHB, moderate plant height, grain yield, and resistance to yellow rust. Eight of them carried *Fhb1* resistance gene and five probably had other FHB resistance QTLs from 'Sumai 3' spring wheat parent or combined minor resistance QTLs from winter wheat cultivars.

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Variations in the D-genome chromosomes of hexaploid/tetraploid wheat crosses analysed utilising cytology and molecular markers

Sriram Padmanaban[a], Peng Zhang[b], Mark W. Sutherland[a], Noel L. Knight[a] and Anke Martin[a]

[a] *Centre of Crop Health, University of Southern Queensland, Toowoomba, Queensland, Australia*
[b] *Plant Breeding Institute, University of Sydney, Cobbitty, New South Wales, Australia*

Sriram.padmanaban@usq.edu.au

Hexaploid bread wheat (*Triticum aestivum* L.) and tetraploid durum wheat (*T. turgidum* ssp. *durum*) are the two main wheat species commercially cultivated in Australia. Interspecific hybridisation between these two wheat species creates pentaploid wheat hybrids, which have unique chromosomal configurations in F_1 hybrids with 14 bivalent of A and B and seven univalent of D chromosomes. The progenies show various chromosome numbers ranging from 2n=28 to 2n=42 in the subsequent F_2 generations based on the D genome loss or retention, and these lines may take a few generations for the stability of D-genome into either hexaploid or tetraploid parent. Understanding the differences in the retention of D-genome chromosomes among different hexaploid/tetraploid crosses will greatly assist breeding programmes to efficiently develop elite bread and/or durum wheat lines for disease resistance, quality enhancement and improving agronomic characters from these pentaploid hybrids. In this study a cross between a doubled haploid hexaploid parent 2-49/W21MMT70_E25 and a tetraploid durum parent 950329 was made and assessed for the retention of D chromosomes in the F_2 generation. Out of 31 lines examined 15 lines retained at least one copy of all seven D chromosomes.

Twenty-five F_3 lines of this population were studied firstly to determine their retention of the seven D chromosomes and secondly to estimate the proportion of A- and B-genome sequences inherited from the bread wheat. Screening with 1,915 DArTseq markers located across the hexaploid genome indicated that more than 48% of the progeny retained all seven individual D chromosomes and a further 44% retained at least six D chromosomes. The remaining 8% had lost either two or three D chromosomes. No significant difference was observed in the overall proportion of A and B genome materials inherited from the bread and the durum wheat. Individual lines, however, had varying proportions of A and B chromosomes inherited from the bread wheat parent. A moderate correlation ($r = 0.587$) was observed between the proportion of A and B chromosomes inherited from bread wheat and the retention of D chromosomes. Three possible translocations or chromosomal deletions were observed on chromosomes 1DL, 2DS and 6DL.

An F_4 generation was established with 25 seeds selected from four lines that retained all the seven D chromosomes. To investigate the behaviour of D chromosomes, F_4 lines were backcrossed with durum parent 950329. Seeds were collected from the selfed F_5, and backcrossed lines are currently being analysed with cytology. This study follows the behaviour of univalent D chromosomes in the 2-49/W21MMT70_E25/950329 cross in a number of generations. Since the identified cross seems to have the potential to retain a substantial amount of D chromosomes in the subsequent generations, it may be suitable for developing improved bread wheat lines that incorporate desirable traits from the durum source.

Influence of individual bread wheat chromosomes on double haploids (DHs) production

Myroslava Rubtsova, Astrid Junker, Kerstin Neumann, Ingo Muecke, Andreas Börner and Thomas Altman

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

rubtsova@ipk-gatersleben.de

Double Haploids (DHs) lines are commonly used in plant breeding programs for the production of homozygous individuals in a single step. DHs applied in marker studies speed up the development of mapping populations and marker/trait associations. DHs facilitate hybrid breeding, and they are useful in fixing traits rapidly in desirable combinations in a line/variety. Breeders can evaluate DH lines with more speed, accuracy and confidence, especially in respect to quantitatively inherited traits such as yield and quality. Androgenic embryo-like structures (ELS) induced from microspores make a perfect model to study embryogenesis and other aspects of plant developmental biology and they are useful as targets for inducing mutation and transformation.

Androgenesis is the most widely effective technology deployed in obtaining DHs and it takes advantage of the large amount of microspores which are produced by a single plant to develop in the production of homozygous lines. Anther culture method included two vital *in vitro* steps: the induction of the androgenic process and the regeneration of haploid/double haploid plants. It has been demonstrated that overall wheat haploid plant production from anther culture is controlled by at least three different and independently inherited traits (Lantos et al. 2013) and is determined by a number of factors such as: ELS induction rate, their regeneration ability and the ratio of green to albino plants. We investigated a manifestation of these traits in nulli-tetrasomic lines of wheat by growing them in independent conditions: greenhouse and phytotron and we showed that D genome of bread wheat carries factors which have positive effect on stimulation of ELS production, on regeneration capacity of ELS and on formation of albino plants, while on B genome genes which decrease manifestation of these traits are located. After analyzing of introgression lines we draw near to the location of the gene(s) responsible for ELS formation on 7DL chromosome. We observed that absence of 7A chromosome in N7A/T7B line leads to disruption of amount of generative organs (number of anthers and pistils). Therefore we assume that 7A chromosome contains gene(s) required for the early stages of ontogenesis in wheat.

Investigation of drought tolerance in bread wheat mapping population of Plainsman V x Mv Magma in field experiments

Krisztina Balla, Ottó Veisz, Szilvia Bencze, Tibor Kiss and Ildikó Karsai

Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Hungary

balla.krisztina@agrar.mta.hu

Drought is one of the most frequent forms of abiotic stress, representing a great danger to crop production in Hungary and having a severe negative effect on cereal yields. This stress factor is becoming increasingly frequent and can affect wheat plants at any time during vegetative growth. A mapping population consisting of 134 doubled haploid (DH) lines was created from two varieties considered to be drought tolerant/heat sensitive (Plainsman V.) and drought sensitive/heat-tolerant (Mv Magma) in order to study drought tolerance and gain a more detailed understanding of the genetic background. Field experiments were carried out in four consecutive seasons between 2011 and 2014 in Martonvásár under natural rain-fed and irrigated conditions. The object of the experiment was to determine the morphological components and yield production capacity of the DH lines and to prepare QTL analysis based on the phenotypic data matrix of genotypes collected in the natural rain-fed, irrigated and the various platform (osmotic and heat stress) experiments. Variance components of yield related traits showed that the environment had the strongest effect on plant height (PH), chlorophyll content (Chl83), thousand kernel weight (TKW) and reproductive tiller number (RT). PH, Chl83 and ear length (EAL) varied with the seasons to the largest extent, while grain yield (SY), RT and spikelet number (SPN) varied with the water regime. Irrigation had the strongest effect on the examined traits in 2012 (with the warmest and driest spring), when with the exception of grain number/spike, all the other traits (PH, EAL, SPN, RT, TKW, SY, Chl83) were significantly higher in the irrigated treatment than under the rain-fed condition. In 2014 (with the coolest and rainy spring) on the other hand irrigation had a much less effect, only the RT and SY were significantly higher due to the well-watered treatment. In the last two seasons irrigation had a negative effect on the TKW.

The multi-environment and multi-trait QTL analyses were carried out using the GenStat 17 software and the marker linkage map was constructed with JoinMap software. In the DH lines 327 DArT and SSR markers were placed in 27 linkage groups covering 17 of 21 wheat chromosomes, with a total of 1864.9 cM recombination distances. Significant QTL effects were identified for all the traits studied; the number of QTL per trait ranged between 3 (RT) and 10 (AS, average grain number per spike). 65 QTLs of individual traits were identified in the field experiments of 4 years and two water regimes, which were organised into 17 chromosomal regions. Of this 3D, 6B and 7BL showed the most complex effects. 80% of these QTLs were consistent across the environments and explained only a small portion of the phenotypic variance individually. The largest effect QTLs were those showing significant interactions with the environment. 4 of the 17 chromosome regions had significant interactions with the year irrespective to the water regime. Only two chromosome regions – 1BL and 3D – associated with the water regime irrespective to the year. QTLs of osmotic stress response overlapped with the field experiment QTLs in 6 chromosome regions (3B, 3D, 5A, 6AL, 6B and 7BL), to 1 of which (6B) QTLs of heat stress response was also located. Four QTL regions were identified as the most promising and interesting for further research and breeding purposes: 1BL, 3D, 6B and 7BL.

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Infection severity and mycotoxin production of *Fusarium culmorum* in wheat grown at elevated CO_2 level

Szilvia Bencze, Gyula Vida, Ildikó Karsai, Krisztina Balla, Katalin Puskás and Ottó Veisz

Agricultural Institute, Centre for Agricultural Research, HAS, Hungary

bencze.szilvia@agrar.mta.hu

Increasing atmospheric CO_2 levels affect the metabolism, physiology and development of plants resulting in changes in the plant - pathogen interactions. Although the impact of elevated concentration of CO_2 is well-known on plants, much less attention has been paid to the interaction between plants and diseases. The aim of the present work was to determine the influence of CO_2 enrichment on the infection severity of *Fusarium* head blight (FHB) in wheat and on the grain mycotoxin contamination. Three experiments have been conducted in recent years in growth chambers of the phytotron (Exp1, Exp3) and greenhouse (Exp2) in Martonvásár, under slightly varying conditions. Plants of selected winter wheat varieties with different genetic background and various resistance levels to FHB (Mv Regiment – Reg, Mv Mambo – Mam, Mv Emma – Em, Apache – Ap and Ukrainka – Uk) were grown in pots either at ambient (400ppm – NC) or elevated (750ppm – EC) CO_2 level. Two kinds of inoculation were performed with *F. culmorum* to examine the resistance against the spread of the fungus from an inoculated spikelet (single floret inoculation – SFI) and to test the combined resistance to the penetration and spread of the pathogen (whole-spike inoculation – WSI). After maturation in Exp1 and Exp3, the grain yield of infected spikes of selected varieties was used to determine the deoxynivalenol (DON) and the zearalenone (toxin F2) concentrations based on the method of competitive enzyme immuno-assay. It was found that FHB severity was not or very little affected by the CO_2 concentration in the case of SFI as the area under the disease progress curve did not change due to EC in three varieties. A significant rise was only found in Uk, while a decrease was recorded in Reg only in Exp1. WSI resulted in similar infection severity at both NC and EC in four of the five varieties tested. Despite the fact that infection severity seemed to be less influenced by the CO_2 level, the DON content increased considerably in response to EC in the case of SFI in all of the four varieties tested. When ZEA concentration was above the detection limit, it exhibited a similar increasing trend at EC. In WSI, there was no significant change in the DON concentration at EC in two varieties, while an increase was found in Ap and a decrease was recorded in Reg. ZEA content was not detectable in two varieties but in Reg and Ap it exhibited a drop in response to EC.

The results indicate that a future environment with higher CO_2 levels might imply a higher risk of food security as mycotoxin production might increase considerably even when disease severity does not change.

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Detection of mutations in carotenoid genes in a durum wheat tilling population by DHPLC technology

Pasqualina Colasuonno, Maria Luisa Lozito, Agata Gadaleta, Ornella Incerti and **Antonio Blanco**

Department of Soil, Plant and Food Sciences, sect. Genetics and Plant Breeding, University of Bari, Bari, Italy

antonio.blanco@uniba.it

Durum wheat (*Triticum turgidum* L.) is a cereal crop widely grown in the Mediterranean regions; the amber grain is mainly used for the production of pasta, couscous and typical breads. Single nucleotide polymorphism (SNP) detection technologies and highthroughput mutation induction represent a new challenge in wheat genetic breeding to identify allelic variation in large population. The TILLING strategy makes use of traditional chemical mutagenesis to induce mutations detected as single base mismatches in heteroduplex status. Although TILLING has been combined to several sensitive pre-screening methods for SNP analysis, most of them are limited for costs and high-throughput application. Recently, a new low cost and time saving DHPLC protocol has been used in molecular human diagnostic to detect unknown mutations.

In this work, we developed and characterized a new durum wheat TILLING population (cv. Marco Aurelio) at 0.70-0.85% ethyl methane sulfonate concentration (EMS). To investigate the efficiency of the mutagenic treatment, a pilot screening was carried out on 1,140 mutant lines focusing on two target genes (Lycopene epsilon-cyclase, ε-LCY, and Lycopene beta-cyclase, β-LCY) involved in carotenoid metabolism in wheat grains. We simplify the heteroduplex detection by two low cost methods: the enzymatic cleavage (CelI)/agarose gel technique and the denaturing high-performance liquid chromatography (DHPLC). The CelI/agarose gel approach allowed us to identify 31 mutations, whereas the DHPLC procedure detected a total of 46 mutations for both genes. All detected mutations were confirmed by direct sequencing. The estimated overall mutation frequency for the pilot assay by the DHPLC methodology resulted to be of 1/77 kb, meaning a high probability to detect interesting mutations in the target genes. We produced and characterized a new durum wheat TILLING population useful for a better understanding of key gene function. We demonstrated the applicability and efficiency of a new strategy for an efficient detection of induced variability. The availability of this tool together with TILLING technique will expand the polymorphisms in candidate genes of agronomical important traits in wheat.

Detection of leaf rust resistance genes *Lr24*, *Lr25*, *Lr28*, *Lr34* and *Lr35* in winter wheat cultivars

Paweł Cz. Czembor, Magdalena Radecka-Janusik and Piotr Słowacki

Plant Breeding & Acclimatization Institute – National Research Institute, Department of Plant Breeding and Genetics, Radzików, Poland

p.czembor@ihar.edu.pl

Leaf rust caused by the biotrophic fungus *Puccinia triticina* Eriks. is one of the most important fungal disease affecting wheat worldwide. Genetic resistance is the most economical, effective and ecologically sustainable method of controlling the disease. Over 70 leaf rust resistance genes have been characterized. Efficient utilization of genetic resistance relies on an appropriate knowledge of the leaf rust resistance genes and of their effectiveness in different environments. This knowledge would augment our understanding of the durability of the genes and may assist in incorporation of resistance genes in adapted germplasm. Resistance genes can be postulated based on the well-known gene-for-gene concept. However, in recent years closely linked or perfect (derived from a gene sequence) markers have been identified for many of the *Lr* genes.

The aim of the study was the evaluation of molecular markers specificity for five *Lr* genes and their detection in 150 European winter wheat cultivars (WWCC) comprising 83 cultivars registered on national list of COBORU (Research Centre for Cultivar Testing, Poland) and other European cultivars (Germany, France, United Kingdom and Switzerland). The specificity of molecular markers was analyzed on NILs of cv. 'Thatcher' (TcNILs) carrying known *Lr* resistance genes (standard *Lr* differential set). DNA of WWCC and TcNILs was amplified in PCR using specific markers linked to the *Lr* genes: *Lr24* (wPt8845-3, PCR product 370bp), *Lr25* (combination of markers gwm6 – no PCR product and gwm251 – 124bp), *Lr28* ($SCS421_{570}$-1/ $SCS421_{570}$-2 – 570bp), *Lr34* (cssfr5 – 751bp) and *Lr35* (Sr39-F2/Sr39-R3 – 900bp).

All markers were specific on *Lr* differential set comprising 42 lines, i.e. for a given resistance gene above mentioned PCR product (amplified from closely linked marker locus) was detected only in line carrying this gene. Among 150 wheat cultivars amplification of specific markers gave following results:

- *Lr24* was detected in cultivars: Caroll, Desamo, Elixer, Lithium, Memory, Tentation, Waxy and Xantippe
- *Lr28* was detected in cultivars: Addict, Belepi, Capone, Elixer, Ennsio, Eron, Fermi, Gordian, Heros, Ionesco, Kredo, Lear, Matheo, RGT Djoko, Pengar, Speedway, Scout, Tabasco, Terroir, Tobak and Zappa
- *Lr34* was detected only in cultivar Baletka
- *Lr25* and *Lr35* were not detected in WWCC

Contribution of stem reserves to grain weight in wheat under terminal drought

Dejan Dodig[a], Vesna Kandić[a], Jasna Savić[b], Dragana Rančić[b] and Biljana Vucelić-Radović[b]

[a] *Maize Research Institute Zemun Polje, Serbia*
[b] *Faculty of Agriculture, University of Belgrade, Serbia*

ddodig@mrizp.rs

When environmental stress develops during reproductive phases of growth, wheat plants have to rely increasingly on remobilisation of previously stored assimilates to maintain grain filling. The present study was undertaken to assess the contribution of the stem (based on dry weight) and the uppermost internode i.e. peduncle (based on water-soluble carbohydrates) to grain weight under near optimal and terminal drought conditions. In two-year field trials 61 wheat genotypes were used (27 F4:5 families, 17 parents used for the crosses and our 17 current best standards), comparing plants that were defoliated (DP) by cutting off all leaf blades 10 days after anthesis with intact control plants (CP). Estimated contributions of stem and sheath assimilate reserves to grain weight/spike were from 10–54% and from 24–84% in CP and DP plants, respectively. Stem-related traits were among key traits determining stem reserve contribution. Estimated contributions of peduncle (culm and flag leaf sheath) assimilate reserves to grain weight/spike were from 6 to 31 % and from 11 to 45% in CP and DP plants, respectively. In both CP and DP plants a higher contribution was from the leaf sheath than from culm. Traits mostly responsible for discrimination between the genotype groups in DP were flag leaf area, peduncle share in total stem length and biomass of main stem. In general F4:5 families, that had been crossed to combine typical breeding traits such as biomass and yield components, showed better tolerance under moderate stress than standards and parents. These traditional traits also benefit yield productivity and grain filling under non-stress environments. However, a number of traits that are expected to be of specific benefit under severe stress conditions probably need to be targeted. Various aspects of stem anatomy and post-anthesis variation in water-soluble carbohydrates have also been studied in these trials.

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A century of Swiss wheat breeding decreased rooting depth under-well watered conditions but maintained deep rooting in drying soil

Cordula N. Friedli[a], Samuel Abiven[b], Dario Fossati[c], Achim Walter[a] and Andreas Hund[a]

[a]*Institute of Agricultural Science, ETHZ, Switzerland*
[b]*Soil Science and Biogeochemistry, University of Zurich, Switzerland*
[c]*Agroscope IPV,Switzerland*

cordula.friedli@usys.ethz.ch

Improving root architecture is an important aim to adapt plants to reduced water and nutrient availability as well as to enhance carbon sequestration. Specifically, deep rooting is discussed as a promising strategy to improve water uptake under drought. However, in the last century, wheat breeders have dramatically reduced plant height by introducing dwarfing genes into their material during the 'Green Revolution'. These new, semi-dwarf varieties showed reduced lodging under high input and an increased harvest index.
What about the roots? There is limited information about how rooting depth and root biomass was affected by breeders and there are no studies which investigated root architecture of Swiss wheat.
The objectives of our study were to elucidate i) how root architecture of winter wheat changed over 100 years of breeding and ii) how root architecture of winter wheat is influenced by emerging drought during early vegetative and/or during late reproductive development. We examined the 14 most important Swiss bread wheat varieties of the last century and promising modern varieties of the top or first class of bread wheat.
Varieties were grown in our Deep Root Observation Platform (DROP) under controlled conditions. Two water stress treatments were established: early water stress in the vegetative phase and water stress in the reproductive phase. Both water stress treatments were established starting at complete field capacity. Thus, water scarcity in the root zones developed gradually starting from the upper part of the soil column with most intensive rooting. Plants were harvested at flowering and maturity, and root biomass distribution in the columns was determined in 25 cm intervals of 160 cm total below ground depth.
Our preliminary results indicate that reduction in plant height led to a severe reduction in rooting depth under normal conditions. In comparison to well-watered conditions, modern, shorter varieties showed deeper rooting depth under water-stress in the vegetative phase, whereas the rooting depth of old genotypes (released before the 'Green Revolution') decreased. Under water limited conditions, root dry weight reduced strongly for all genotypes compared to root dry weight under well-watered conditions. Modern varieties showed the trend to compensate water scarcity during the reproductive phase by post-anthesis root growth which results in increased rooting depth and higher root dry weight values.

The effect of dwarfing genes on coleoptile length of bread wheat under conditions of osmotic stress

Anastasiia Honcharova

Plant Breeding and Genetics Institute – National Center of Seed and Cultivar Investigation, Ukraine

goncharovanastya@mail.ru

Drought is a major problem throughout the world, which seriously affects crop yield and quality.

Foreign researchers have already pointed out that the dwarfing genes (*Rht*) selected from bread wheat genotypes play great role in tackling water scarcity. Genes *Rht-B1* and *Rht-D1b* reduce the length of coleoptile, leaves and stems. *Rht8* reduces plant height without affecting coleoptile length (Landjeva et al., 2011). Coleoptile length affects drought tolerance of bread wheat since longer coleoptile allows deeper sowing that contributes to more efficient water absorption.

The aim of our study was to investigate the influence of dwarfing genes implied on drought tolerance of bread wheat in germination phase. The study was conducted on the analogue lines of bread wheat varieties Kooperatorka, Odes'ka 3, Odes'ka 51, Stepniak. These varieties were created by Hanhildinym V.V. and Motsnym I.I. and colleagues at PBGI – NCSCI. Analogue lines differ in dwarfing gene alleles. Alleles of *Rht* genes in analogue lines were identified in previous studies (Chebotar et al., 2009).

We studied the effect of water deficit on coleoptile length. Water deficit was provoked by using a 15% polyethylene glycol 6000 (PEG 6000) solution (Landjeva et al., 2011). Distilled water was used as control. 7-day draught stress was applied from the beginning of germination.

Tall variety Kooperatorka (*rht*) developed longer coleoptiles than its analogue lines Kooperatorka K-90 (*Rht8c*) and Kooperatorka K-70 (*Rht8c, Rht-B1e*) 12,2 and 37,0% ($P < 0,01$), respectively. Under osmotic stress conditions, Kooperatorka K-90 and Kooperatorka K-70 developing shorter coleoptile resembled to the recurrent form Kooperatorka 26,1 and 59,6% ($P < 0,01$), respectively. Coleoptile length difference between Odes'ka 3 (*rht*) and Odes'ka 3 K-75 (*Rht8c, Rht-B1b*) was 27,4% ($P < 0,01$) under control conditions and 61,1% ($P < 0,05$) under stress conditions. Coleoptile length difference between Odes'ka 51 (*Rht8c*) and Odes'ka 51K-73 (*Rht8c, Rht-B1e*) was 15,6% ($P < 0,01$) under control conditions and 31,8% ($P < 0,05$) under osmotic stress conditions. Under control conditions, coleoptile length of Stepniak (*rht*) was greater than the coleoptile length of the analogue lines Stepniak 2 (*Rht8x*), Stepniak 3 (*Rht8c*) and Stepniak 2K (*Rht8c, Rht-D1b*) of 1,3, 12,2 and 18,5% ($P < 0,01$), respectively. Under stress conditions, Stepniak variety developed longer coleoptiles compared to Stepniak 2, Stepniak 3 and Stepniak 2K at 10,1, 20,6 and 23,6% ($P < 0,05$), respectively.

Our studies revealed that dwarfing gene alleles of bread wheat genotypes is important for reducing coleoptile length under osmotic stress conditions.

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Variability of oil and tocopherol content in wheat

Nikola Hristov, Novica Mladenov, Ankica Kondić-Špika, **Ana Marjanović-Jeromela**, Nada Grahovac, Bojan Jocković, Vladimir Aćin, Milan Mirosavljević and Vojislava Momčilović

Institute of Field and Vegetable Crops, Serbia

nikola.hristov@ifvcns.ns.ac.rs

Wheat is one of the most widespread plant species, primarily because of its adaptability and diverse uses. Increased application of wheat oil in food, beauty and pharmaceutical industries has led to an increased importance of oil content as an indicator of quality. Wheat oil has beneficial effects on human health due to its high content of tocopherols and polyunsaturated fatty acids. The most important chemical traits of tocopherols are their antioxidant activities and biological function in vitamin E activities. The aim of this study was to determine the variability of oil and tocopherols content in 24 genetically divergent wheat cultivars from different selection cycles during a two-year period. The oil was extracted from wheat bran obtained by using laboratory mill MLU 202. The classic Rushkovsky method was used to determine the oil content, while tocopherols (α, β γ) were simultaneously determined using hexane extraction and liquid chromatography with detection of fluorescence. The average oil content during two years ranged from 4.1% to 4.3%, while the minimum and maximum values ranged from 3.2% to 4.9%. The coefficient of variation (CV) value was 8.6%. The average content of α tocopherols during two years ranged from 9.1 to 13.1 mg/kg bran (min. and max. values: 3.8-18.5 mg/kg bran), β tocopherols 6.5-8.2 mg/kg bran (min. and max. values: 2.9-11.1 mg/kg bran) and γ tocopherols 31.1-53.0 mg/kg bran (min. and max. values: 29.2-67.4 mg/kg bran). The CVs for the contents of α, β and γ tocopherols were 8, 4.6 and 3.4%, respectively. The effect of genetic factors and environment (and their interaction), were highly significant for all analysed parameters, and highly significant correlations were found between them (correlations varied from 0,871** between oil content and γ tocopherols to 0.990** between α and β tocopherols). Cluster analysis showing the degree of similarities was used to group the analysed varieties into clusters, which can serve as the basis for the selection of parental components to further the wheat breeding for higher oil content and quality. The results obtained from wheat bran have shown lower oil content and higher content of γ tocopherols in relation to the direct extraction from the wheat germ. Varieties with higher oil and tocopherol content can be used as a good raw material for whole-wheat products with important benefits to the human health.

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Rethinking the wheat seed system in Pakistan: fast track delivery of new genetic gains to farmers

Krishna D. Joshi[a], Attiq U. Rehman [a], Ghulam Ullah [a], Mahreen Zahara[b], Akhlaq Hussain[c], Muhammad Suleman [b], Mian F. Nazir [b], Ehsan Elahi [b], Muhammad Khan[d] Abid I. Da[re], Amanullah Baloch [a] and Muhammad Imtiaz [a]

[a]*International Maize and Wheat Improvement Center, Islamabad, Pakistan,*
[b]*National Rural Support Programme, Islamabad, Pakistan,*
[c]*Ex Director General Federal Seed Certification and Registration Department, Islamabad, Pakistan*
[d]*Department of Agriculture Extension, Peshwar, Pakistan*
[e]*Engro Fertilizers Ltd. Lahore, Pakistan*

k.d.joshi@cgiar.org

Wheat is a strategic crop for food security in Pakistan. Wheat breeding, variety testing, and varietal release are controlled by public organizations, and the formal seed system allows for the supply of only certified seed. Moreover, farmers lack knowledge about the genetic attributes or agronomic performance of new varieties, due to a lack of systematic popularization efforts across the country. Market demand for wheat seed is determined by popularity rather than varietal merit. Old and established varieties are most popular and the formal seed sector is promoting such varieties to reduce operating costs, ignoring the potentially catastrophic threat of rust epidemics overcoming the resistance in such older varieties.

In 2014, Pakistani farmers were growing 8-10 years old wheat varieties compared to 1997 when weighted age of wheat varieties was 6-8 years indicating a slowdown in varietal replacement rate over 17 years period. Farmers growing older improved varieties lose out on gains from improved yield potential or better disease resistance or better grain and nutritional qualities of newly released varieties.

The slowness and inflexibility of seed production and delivery systems also limits active participation of private companies or farmers seed production groups. Private companies depend on officially released varieties and the limited quantity of source seed from public sector organizations, further constraining germplasm development and fast delivery of genetic gains.

This paper highlights simple, rapid, innovative and cost-effective approaches to fast-track germplasm evaluation and the deployment of genetic gains by more quickly and effectively delivering new wheat varieties to farmers, with focus on smallholder farmers and women alike.

Microsatellite loci associated with heading time in Ukrainian bread wheat varieties

Olga Kolesnyk and Aleksandr Khokhlov

Department of General and Molecular Genetics, Plant Breeding and Genetics Institute (PBGI) – National Center of Seed and Cultivar Investigation, Ukraine

emerald-olga@ukr.net

Nowadays, a great progress in a conventional breeding is enhanced by introgression of new molecular marker technology that facilitates the traditional breeding process (Brbaklic et al., 2013). Heading time (HT) is one of the critical traits for the adaptation of bread wheat (*Triticum aestivum* L.) to diverse climatic environments and the cultivation in various regions and cropping seasons. The adaptability of wheat to a wide range of environments has been favored by allelic diversity in genes regulating growth habit and photoperiod response (Zanke et al., 2014). In our research we determined genetic diversity and phylogenetic relationship among a group of 48 bread winter wheat varieties (250 bread wheat lines of different varieties) of PBGI breeding revealed by SSR markers, the variance of their HT trait which was determined in the field conditions during three growing seasons and also potential SSR markers associated with these trait. The main goal of this work was focused on finding marker trait associations (MTAs) in the investigated varieties. Thus 13 MTAs were shown to be stable in three growing seasons, namely alleles *Xgwm357*$_{123}$, *Xgwm357*$_{128}$, *Xtaglgap*$_{215}$, *Xtaglgap*$_{238}$, *Xgwm325*$_{128}$, *Xgwm325*$_{148}$, *Xgwm325*$_{150}$ and *Xwmc405*$_{222}$ were found to be significantly associated with the later date of HT while alleles *Xgwm357*$_{116}$, *Xtaglgap*$_{218}$, *Xgwm155*$_{143}$, *Xgwm325*$_{115}$, *Xgwm325*$_{146}$ showed to be significantly associated with the earlier date of HT. Additionally during two growing seasons 12 alleles were significantly associated with the later date of HT while 10 alleles showed association with the earlier date of HT. The HT values associated with presenting of contrast alleles pairs in seven different loci are shown in Fig. 1.

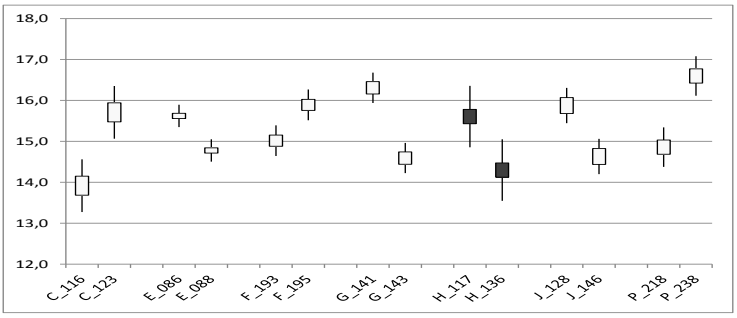

Fig. 1. HT values associated with presenting of contrast alleles pairs in seven different loci, means for 3 seasons; X-axis shows days from 1 May when approximately half of ears in a plot have half emerged from the flag leaf; Y-axis shows alleles pairs in seven different loci: C – *Xgwm357*-1A; E – *Xgwm3*-3D; F – *Xgwm165/1*-4A; H – *Xgwm389*-3B; J – *Xgwm325*-6D; P – *Xtaglgap*-1B. The numbers behind the letters reflect the sizes of alleles (bp). Bars span range Mean± (Sd/2) and whiskers – range Mean ± (LSD_{05}/2). Solid black bars indicate pair with inter-allelic differences stable over the years, but with certainty below p = 0.05

The findings of new MTAs, beside well-known *Eps*, *Ppd* and *Vrn* genes, could have a great impact to shortening of HT, which have the preferences in our environmental conditions. Confirmation of MTAs in our material opens new opportunities for using the pointed microsatellite markers for MAS.

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Effect of *Ppd* alleles on yield performance in wheat

Ankica Kondić-Špika[a], Dragana Trkulja[a], Nikola Hristov[a], Ljiljana Brbaklić[b], Borislav Kobiljski[b] and Simon Griffiths[c]

[a] *Institute of Field and Vegetable Crops, Novi Sad, Serbia*
[b] *Biogranum, Novi Sad, Serbia*
[c] *John Innes Centre, Norwich, UK*

ankica.spika@nsseme.com

In agro-ecological conditions of Serbia and the whole region of South-Eastern Europe high temperatures can occur very early in the spring. When high temperatures combines with drought conditions during the spring and early summer it causes significant yield losses in wheat production. One of the goals of breeding programs is to create varieties with early heading and flowering but in the same time with late maturity in order to prolong grain filling and increase the yield. It can be achieved with specific allelic combinations of genes that influence wheat phenology and adaptability, such as *Ppd, Vrn* and *Eps* genes.

The aim of this study was to use specific genetic material (NILs of cv. Paragon) in order to determine the effect of different *Ppd* alleles on yield performance of wheat genotypes in our environmental conditions. The trial was conducted at the experimental field of the Institute of Field and Vegetable Crops in Novi Sad (IFVCNS), Serbia at the location of Rimski sancevi (45°20`N, 19°51`E). The material consisted 10 modern Serbian wheat varieties (set 1) and 54 NILs of cv. Paragon. Two groups of the NILs had introgressed single (set 2) and double (set 3) insensitivity and early *Ppd-1* alleles, while the third group formed the NILs with single, double or triple introgressed *Ppd-1* null, knock-outs or late alleles (set 4). The genotypes were sown in the plot size of 2 m^2 (2x1) with six rows per plot in three replications. The following traits were measured during the two growing seasons (2014 and 2015): whole stem length (cm), peduncle length (cm), spike length (cm), spikelet number per spike, grain number per spike, TGW – thousand grain weight (g), and grain yield (g).

The results revealed differences between our well adapted cultivars and NILs with specific combinations of *Ppd* alleles regarding the yield and yield related traits in our agro-climate conditions. All sets of NILs had significantly higher number of grains (from 13-19% in both seasons), but also lower TGW (10-29.5% in 2014 and 10-26.8% in 2015) in relation to our cultivars (set 1). Regarding the grain yield there was a significant difference in the effect of *Ppd* alleles in two growing seasons. In 2014 with better temperature and moisture conditions from March to July, the sets 2 and 3 had higher grain yield (5.9% and 4.0%, respectively), while the set 4 had significantly lower grain yield then our cultivars (31.6%). In the less favourable season of 2015 all the sets of NILs had 10.4-37% lower grain yield then our cultivars.

PCA of collected data showed a clear separation between sets 1 and 4, regarding all traits measured in both growing seasons. Set 3 was the closest to our well adapted cultivars, while set 2 was in the middle of 3 and 4. Detailed statistical analysis will reveal which allelic combination can lead to creation of new wheat cultivars with improved yield performance.

Acknowledgement

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The influence of medium composition on embryogenic callus induction and plant regeneration from mature embryos of wheat cultivars with various resistance to *Parastagonospora nodorum*

Lidia Kowalska[a] and Edward Arseniuk[a]

[a]*Plant Breeding and Acclimatization Institute, National Research Institute, Department of Plant Pathology, Poland*

l.kowalska@ihar.edu.pl

Glume and leaf blotch is a fungal disease of wheat (*Triticum aestivum* L.) elicited by a necrotrophic fungus *Parastagonospora nodorum.* It is a serious pathogen of wheat worldwide, which by reducing assimilative area of plants affects adversely quantity and quality of grain yield. Among wheat cultivars complete resistance to *P. nodorum* and other species of *Parastagonospora* is not encountered. Low efficiency of breeding programs is associated with polygenic conditioning of resistance to the pathogen. Inheritance of wheat resistance to *P. nodorum* is considered to be additive. Since recurrent resistance breeding of wheat to this pathogen is slow and with little effect. Alternative approaches have been exercised. The latter concern primarily biotechnological tools e.g. somatic embryogenesis and androgenesis. This research has focussed on these methods.
The first step has been directed to improve embryogenic callus induction and plant regeneration from wheat mature embryos. For the purpose six winter wheat cultivars with various resistance to *P. nodorum* were tested. At the beginning effects of three auxins [2,4 dichlorophenoxyacetic acid (2,4-D); 3,6-dichloro-o-anisic acid (dicamba); 1-naphthaleneacetic acid (NAA)], and the effect of maltose vs. sucrose were evaluated. The percentage of embryos producing an embryogenic callus, non-embryogenic callus and plant regenerating embryos rations were employed to assess phenotypic response of wheat genotypes in the *in vitro* culture. The results demonstrated relatively high embryogenic potential of all winter wheat cultivars used in the study. Inducing media supplemented with dicamba and sucrose were most suitable for embryogenic callus formation. However, the highest efficiency of plant regeneration was obtained on medium without phytohormones. The results of this study will increase knowledge about tissue culture response of wheat and bring closer the use of biotechnological methods to improve Parastagonospora nodorum blotch resistance of the species along with the shortening of breeding cycle to this disease.

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Genome-wide association mapping for phenolic acids concentration and composition in a collection of tetraploid wheats

Barbara Laddomada[a], Domenica Nigro[b], Antonella Pasqualone[c], Laura Nunzia Delvecchio[c], **Rosanna Simeone[b]**, Agata Gadaleta[b], Miriana Durante[a], Leone D'Amico[a], Giovanni Mita[a] and Antonio Blanco[b]

[a]*Institute of Sciences of Food Production, National Research Council, ISPA - CNR, Italy*
[b]*Department of Soil, Plant, and Food Sciences, Plant Breeding Section, University of Bari, Italy*
[c]*Department of Soil, Plant, and Food Sciences, Food Science Section, University of Bari, Italy*

rosanna.simeone@uniba.it

Phenolic acids are major components of plant cell walls in wheat and have important implications on human health as antioxidants with anti-tumor activity. They occur in wheat grains as: i) soluble free phenolic acids; ii) soluble conjugated phenolics bound to low molecular mass components such as saccharides or organic acids; iii) insoluble bound forms of phenolics, linked to polymers of the plant cell wall (Li et al., 2008). Despite their high value for human health, few studies have been carried out on the genetics of phenolic acids in durum wheat.

The genetic variability of phenolic acids composition and concentration was investigated, over two years, in a set of 111 tetraploid wheat genotypes, belonging to seven *Triticum turgidum* L. subspecies, including cultivars, landraces and wild accessions. Regions attributable to individual phenolic acids and total phenolic acids concentration were identified through a genome wide association study (GWAS).

A total of six phenolic acids were identified by DAD-HPLC analysis across the 111 wheat genotypes, namely: ferulic, sinapic, *p*-coumaric, vanillic, syringic and *p*-hydroxibenzoic acids. The amount of total bound phenolic acids ranged from 341 to 1700 μg g^{-1} of whole-meal flour, with a mean value of 800 μg g^{-1} (Laddomada et al, 2016). The soluble free fraction (measured spectrophotometrically after Folin Ciocalteu reaction) ranged from 1280 to 3150 μg g^{-1} as ferulic acid equivalents.

The effects of genotype, year and year × genotype were estimated by ANOVA and resulted significant for all phenolic acids. The ratio of genotypic variance to total variance was moderately high suggesting that a breeding approach could be considered to increase phenolic acids concentration in durum wheat.

The GWAS revealed a total of 29 significant marker-trait associations (MTA), identifying eight quantitative trait loci (QTL) associated with phenolic acids content. The highest number of MTAs was identified on chromosome 7A, where one QTL region was associated with phenolic acids content, while the lowest number of MTAs was detected on chromosomes 3A and 5B, where only one MTA identified a single locus. Conservation of synteny between SNP marker sequences and the annotated genes and proteins in *Brachypodium distachyon*, *Oryza sativa* and *Sorghum bicolor* allowed the identification of two QTLs coincident with two different candidate genes.

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Genetic variation of Asian wheat revealed by HMW glutenin and maturity

Sukyeung Lee, Yu Mi Choi, Do Yoon Hyun, Sejong Oh,
Myung-chul Lee, Hocheol Ko, On Sook Hur

National Agrobiodiversity Center, National Institute of Agricultural Science,
Rural Development Administration, South Korea

reset00@korea.kr

Glutenin is a key factor for mixing and bread baking quality of wheat flour. In particular, the relationship between High Molecular Weight glutenin subunit composition and bread making quality was established by Payne (1987) as *Glu-1* scoring system. This score assigned to each identified *Glu-1* allele makes it possible to predict bread baking quality (X.Shan et al., 2007). Early maturity is one of the most important cultural characteristics of Korean wheat varieties because of rice-wheat dual cropping system. This study is to clarify the genetic variation of Asian wheat collection of landraces and breeding lines originated from Japan, China, India, Mongolia, Kazakhstan, Uzbekistan, and Tajikistan, as a part of follow-up research of large-scale glutenin analysis of wheat collection. Using 1,380 accessions maintained in National Agrobiodiversity Center, NIAS, RDA, Korea, HMW glutenin subunit allelic composition and the maturity date were investigated. Useful accessions having preferable *Glu-1* allelic composition and early maturity were selected to enhance the utilization of genetic resources to Korean wheat breeding. Whereas *Glu-A1c(null), Glu-B1b(7+8)* and *Glu-D1a(2+12)* allele were the most frequent among Asian wheat in this study, *Glu-B1i(17+18)* allele was the most frequent in Indian germplasm. Chinese accessions also had a distinctive characteristic compared to other countries. The composition of *Glu-A1a*, *Glu-B1b* and *Glu-D1d* alleles, which are advantageous for bread baking quality, was especially high. In the evaluation of *Glu-1* score, 37 accessions achieved 10 points and 118 accessions had *Glu-D1d* allele, essential for the fullmark. China (46 samples) and India (24samples) were the most frequent origin of those accessions. Among them, 13 accessions were also matured before early June, suitable to Korean cropping system. Nine accessions (K045274, K140966, K141099, K141107, K141118, K141121, K151941, K162487, K162532) from China had extremely early maturity, ripened even in late May. These genetic resources having good *Glu-1* allelic composition and early maturity are expected to be able to be widely used for Korean wheat breeding system.

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Integration of wild genetic resources with high-throughput genotyping to breed for drought tolerance in durum wheat

Stuart J. Lucas[a], Ayten Salantur[b], Selami Yazar[b] and Hikmet Budak[c]

[a] *SU Nanotechnology Research and Application Centre, Sabanci University, Turkey*
[b] *Biodiversity and Genetic Resources, Field Crops Central Research Institute, Turkey*
[c] *Plant Sciences and Plant Pathology, University of Montana, USA*

slucas@sabanciuniv.edu

Durum wheat (*Triticum turgidum* L. subsp. *durum*) is one of the earliest crops to be domesticated, and continues to be a major crop worldwide. However, climate change threatens to increase the impact of drought and other abiotic stresses on durum production in many of its traditional cultivation areas. Due to domestication and breeding activities, the tetraploid (AABB) *T. durum* genome is thought to have lost some genes that can contribute to drought tolerance. In this study, a diverse hybrid population was created using 19 different wild tetraploid wheat accessions by crossing with 12 elite durum wheat cultivars and advanced lines. The progeny were back-crossed to their durum parents for one generation. After selfing for four further generations, a total of 800 individuals (40 spikes each from 20 different crosses) were planted in single rows. From the resulting F_5 population, 500 lines were selected and used for preliminary yield trials at the F_6 generation.
In order to assess the genetic variation within the F_6 population, selected individuals were genotyped using the Axiom Wheat Breeder's Genotyping array (Affymetrix). As this microarray was designed primarily for use with European hexaploid wheat cultivars, a data analysis procedure was developed and validated for tetraploid wheat genotyping using this system. Over 11,000 (33.4%) of the SNPs on the array were found to be polymorphic within the F_6 hybrid population. These polymorphisms were correlated with physiological traits associated with drought tolerance such as coleoptil length and anthocyanin accumulation. These genotyping data will be helpful to identify loci associated with drought tolerance and select promising genotypes for the durum wheat breeding program.

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QTLs for partial resistance to *Zymoseptoria tritici* in the cultivated durum wheat germplasm investigated by means of a high-density SNP array

Marco Maccaferri [a], Karim Ammar [b], Mohamed Salah Gharbi [c], Seyed Mahmod Tabib Ghaffary [d], Talebi Reza [d], Gert Kema [d], Roberto Tuberosa [a]

[a] *Department of Agricultural Sciences (DipSA), University of Bologna, Bologna, Italy*
[b] *CIMMYT, 56134 Texcoco, Mexico*
[c] *Institut National de la Recherche Agronomique de Tunis, Tunis, Tunisia*
[d] *Plant Research International B.V., Wageningen, The Netherlands*

marco.maccaferri@unibo.it

Durum wheat production in the Mediterranean basin is plagued by fungal diseases, particularly Septoria tritici Blotch (STB). The pathogen's high genome plasticity and specialization features (bread vs. durum wheat) hinder the identification and exploitation of resistance genes across diverse growing areas. A more efficient breeding exploitation of the quantitative variation for STB response present in the cultivated germplam can be envisaged by means of precise molecular investigation tools such as high-density maps of transcript-associated SNPs, genome-wide association analysis (GWAS) and multiple mapping populations analysis. A durum panel of 183 accessions of diverse origins was subjected to field evaluation in Tunisia (three years) and Italy (two years). Seedling inoculation assays were carried out for 16 isolates and genotyping included Illumina 90K SNPs and DArT uniquely mapped in a tetraploid consensus map. While the on-site heritability of field STB responses is rather high (0.78-0.96 in Tunisia and 0.66-0.68 in Italy), accession's STB responses strongly differed between Tunisia and Italy. A GWAS scan based on 13,823 informative SNPs revealed the presence of 21 strong, nominal QTLs ($P \leq 0.001$ marker-wise or Bonferroni-corrected experiment-wise significance $P \leq 0.05$), 10 of which were mostly expressed in the Tunisian environments and 11 in Italian environments. Presence of major QTLs effective in both Countries was minimal. Additional 85 associations ($P \leq 0.01$ marker-wise) were considered as putative QTLs. Based on high-density SNP mapping, most of the QTLs were precisely defined by multiple-associated SNPs within significance intervals of 1.7-5.4 cM. The favorable allele distribution within and among major germplasm breeding groups and the co-location with known *Stb* genes from bread wheat will be further discussed. GWAS-QTLs for partial resistance in the elite germplasm can be further considered for environment-specific breeding selection based on traditional MAS and/or genomic selection.

Pch2 eyespot resistance genes in the double haploid lines of winter wheat (*Triticum aestivum* L.)

Maciej Majka[a], Michał Kwiatek[a], Halina Wiśniewska[a], Jolanta Belter[a], Magdalena Gawłowska[a], Tadeusz Adamski[a], Tadeusz Drzazga[b] and Bogusława Ługowska[c]

[a] *Institute of Plant Genetics of the Polish Academy of Sciences, Poland*
[b] *Malopolska Plant Breeding Company Ltd.*
[c] *Danko Plant Breeding Company Ltd.*

mmaj@igr.poznan.pl

Eyespot is one of the most dangerous stem base diseases of cereals caused by two fungal species: *Oculimacula yallundae* and *Oculimacula acuformis*. The pathogen interfere with the movement of water and nutrients through the stems and leads to lodging of plants and therefore significant yield reduction even up to 50 %. Use of cultivars resistant to eyespot disease is the most economic and environmental friendly way to minimize economic loss of wheat. Therefore, an incorporation of genes for resistance to eyespot is an important aim of varietal improvement. So far only two eyespot resistance genes: *Pch1* and *Pch2* have been characterized and markers made available to plant breeders. *Pch1* originated from *Aegilops ventricosa* is reported as the most effective source of resistance to eyespot, however the chromatin segment of this wild relative of wheat introduce also undesirable traits determining yield reductions. The *Pch2* gene, originated from wheat variety Capelle Desprez, located on chromosome 7AL, can constitute an alternative for maintenance the resistance without negative influence on the agriculturally important traits. Nevertheless the *Pch2* confers a moderate durable resistance and is adequate where disease pressures are not too high.
The main purpose of this study was to evaluate the presence of the molecular markers (*Xcfa2040* and *Xwmc346*) localized nearby on the genetic map of chromosome 7A of wheat in the double haploid lines of winter wheat. The double haploid lines are the last stage of the long-term project which was performed for breeding acceleration of wheat. The plant material (38 lines of wheat) was derived from the breeding lines, originated from: F_3 SMH8592 x KBH4942/05; F_2 KBP0916 x POB32408; F_2 KBP0916 x Jantarka and F_2 KBP0916 x STH9014 (respectively: 30; 14; 28 and 26 plants), which were characterized by the higher tolerance to eyespot. We obtained 439 double haploid lines whereas 22 lines carried eyespot resistance genes. The first step was to cross the selected breeding lines of wheat carrying resistance genes for eyespot what was confirmed with molecular markers. Afterwards selected recombinants were crossed with maize to obtain haploid lines, which were also selected with MAS (markers assisted selection). Finally, after colchicine treatment, the double haploid lines were induced to obtained stable and resistant wheat lines. In breeding, the use of doubled haploids (DH) system together with molecular markers facilitates the identification of linkage between a marker and a trait of interests, and shortens the selection of recombinants.

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Finding new winter wheat ideotype for Southeast Pannonian region

Sonja Marić[a], Ivana Rukavina[b], Sonja Petrović[a], Sunčica Guberac[a], Andrijana Rebekić[a], Tihomir Čupić[c] and Vlado Guberac[a]

[a]*Faculty of Agriculture/Department of Plant Production, University of J.J. Strossmayer, Croatia*
[b]*Croatian Centre for Agriculture, Food and Rural Affairs, Institute for Seeds and Seedlings, Croatia*
[c]*Agricultural Institute Osijek/Department for Breeding and Genetics of Forage Crops, Croatia*

smaric@pfos.hr

Variability of Croatian winter wheat cultivars (*Triticum aestivum* L.) was estimated using 22 morphological characteristics according to UPOV wheat guideline for DUS testing. Cultivars were selected according to registration year (1983 to 2010) and significance in production. Field experiments were set up at locations Osijek 45°32'N and 18°44'E (main location) and Klisa 45°46'N and 18°1'E (reserve location). At both locations cultivars were sown by randomized block design with two replications and plot size was 6.25 m². Each plot included 200 plants / m², a total of 1250 plants per basic plot. The average Dice similarity coefficient was 0.371, ranging from 0.085 to 0.667. Significant variability of 6.21% between registration year and varieties in the breeding programs according to period was determined as well as significant variability of 3.10% between breeding programs. The UPGMA clustering divided cultivars into four main clusters. Correlation coefficient of similarity matrix and dendrogram was 0.68**. Genetic similarity of tested varieties based on morphological data showed clustering by the cultivar type and in some clusters by the origin. According to ANOVA high variation between cultivars per registration period was expected, since genetically different parents and different selection criteria were used in breeding process. Significant, but much lower proportion of the variability between breeding programs can be linked to the fact that they relatively often use similar or partly shared parental components during the process of creating a new variety and in very similar agro-climatic conditions. Analysis of morphological characteristics gave a clear insight into the existing diversity in terms of breeding centres and year of registration and directs towards the most diverse genotypes that can be used as parental lines for a new selection cycle.

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Identification and validation of reference genes for analysis of wheat (*Triticum aestivum* L.) genes expression under drought stress

Michał Nowak[a], Karolina Dudziak[a] and Krzysztof Kowalczyk[a]

[a] *Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin, Poland*

michal.nowak@up.lublin.pl

Nowadays water deficit is a big challenge to agricultural researchers and plant breeders. Drought is one of the most common environmental stresses with strong impact on plant development. Unfavorable environmental conditions lead to an increase of the level of reactive oxygen species (ROS) in plant cells what, in turn, causes activation of different defensive mechanisms on both: enzymatic activity and genes expression levels. Quantitative PCR (qPCR) is currently one of the most sensitive techniques for analysis of gene expression and description of specific gene expression patterns. However, relative analyses of gene expression by means of qPCR requires normalization of variability, using one or several reference gene(s) (also known as housekeeping genes). A proper selection of reference genes is one of the crucial steps of gene expression analysis experiment design.

The aim of the presented study was selection and validation of the putative reference genes, suitable for analysis of genes expression in common wheat (*Triticum aestivum* L.) seedlings subjected to drought. As plant material, a population of 'Janetzkis Probat' (JP) x 'Saratovskaya' 29 (S29) wheat inter-varietal single chromosome substitution lines was used. S29 is drought tolerant and JP is a drought sensitive wheat cultivar. Seedlings of analyzed wheat plants were grown 5 days in MS (Murashige & Skoog) medium in controlled hydroponic conditions. The drought stress was induced by 10% PEG (polyethylene glycol) addition to the medium. Total RNA was extracted from seedlings using Trizol (Ambion) at 3 time points (after 1, 3 and 6 hours). Plants grown in medium without PEG were used as a control forms. RNA concentration and purity was measured spectrophotometrically using NanoDrop and cDNA synthesis was performed using the commercial reagents kit. Obtained cDNA was used as a template for qPCR. For analysis ten putative reference genes were selected. Within them five were typical, universal reference genes used in plant gene expression analysis experiments, encoding actin, tubulin, ubiquitin, translation elongation factor and glyceraldehyde 3-phosphate dehydrogenase. The remaining five genes were selected on the basis of results of *in silico* analysis by means of RefGenes tool in Genevestigator database. This analysis allowed for identification of genes with most stable expression during water deficiency in wheat tissue. Primers for qPCR were designed using PrimerBLAST tool. Absolute analysis of expression of selected genes in all analyzed plants allowed for identification of the gene with most stable expression in experimental conditions. Selected reference gene will be useful for different experiments based on qPCR analysis of genes expression in wheat tissue derived from hydroponic culture and subjected to drought stress.

1BL/1RS translocation effects on efficiency of wheat DH lines production

Piotr Ogrodowicz, Tadeusz Adamski, Maria Surma, Karolina Krystkowiak, Anetta Kuczyńska, Krzysztof Mikołajczak and Renata Trzeciak

Biotechnology Department, Institute of Plant Genetics of the Polish Academy of Sciences, Poznan, Poland

pogr@igr.poznan.pl

Hexaploid wheat (*Triticum aestivum* L.) with a segment of the 1R rye chromosome translocated onto the long arm of the 1B chromosome (1BL/1RS) are widely used in breeding programs due to their resistance to several types of biotic stress and greater yield potential. However, the presence of the translocation is associated with the poor bread-making quality. It was found that the 1BL/1RS translocation has enhanced haploid production and green plant regenerated in the anther culture.

The aim of the study was to examine whether the wheat-rye translocation affects the efficiency of the wheat doubled haploids production via wheat × maize crosses.

Materials for the studies covered winter wheat lines derived from five cross combinations varied in the presence of 1BL/1RS translocation. In each population ten lines possessing 1BL/1RS and ten lines without translocation were distinguished based on analysis of molecular markers. Totally, 100 lines were subjected to the studies. Wheat lines were crossed with maize and the standard procedure was applied as described by Laurie and Bennett (1988). Spikes of wheat were manually emasculated and pollinated with fresh pollen of maize. Maize cultivar Waza was the pollen donor. Pollinated spikes were treated with 2,4-dichlorophenoxyacetic acid (2,4-D). Immature embryos were dissected from seeds 15–18 d after pollination and cultured in vitro on B5 medium in tubes. Traits associated with efficiency of haploid production were observed: number of pollinated spikelets, number of embryos developed and percent of haploid plants obtained. The data were statistically elaborated by the use of uni- and multivariate methods. Among studied populations, in two cases, lines carrying the 1BL/1RS translocation achieved better performance than lines belonging to the subgroup without translocation. In other cases there were no significant differences between efficiency of haploid production between these subgroups. The obtained results suggest that the presence of 1BL/1RS translocation in wheat genome can increase efficiency of haploid production by wheat × maize crossing.

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Looking for *TaCKX* genes associated with grain yield

Agnieszka Onyśk[a], Maja Boczkowska[a], Izabela K. Rajchel[a], Waclaw Orczyk[b] and Anna Nadolska-Orczyk[a]

[a] *Department of Functional Genomics, Plant Breeding and Acclimatization Institute – NRI, Poland*
[b] *Department of Genetic Engineering, Plant Breeding and Acclimatization Institute – NRI, Poland*

a.onysk@ihar.edu.pl

The *CKX* genes belong to a family that is represented in various plant species. The genes encode cytokinin dehydrogenase enzyme (CKX), which irreversibly degrades cytokinins. Expression of the genes as well as cytokinin distribution are spatially and temporally regulated during plant development. We documented that RNAi silencing of the selected, barley *HvCKX* genes influenced grain yield (Zalewski et al. 2010, 2012). Moreover, expression patterns of these genes might indicate their role in growth and reproductive development (Zalewski et al. 2014). The aim of this research is to prove correlation between the level of expression of some of *TaCKX* genes in selected organs/tissues of breeding lines of wheat and grain yield.

Experimental material was collected from two mapping populations (Quarrie i in. 2005, Cuthbert i in. 2008) and two Polish Plant Breeding Seed Companies (PB Strzelce, Ltd., PB Danko). The breeding lines were closely related, however differed in their yield. Total RNA was isolated from seedling roots, leaves, inflorescence and kernels: 7 DAP (days after pollination) and 14 DAP. Isolated RNA was transcribed into cDNA and used for quantitative RT-PCR with PCR starters designed for ten known *TaCKX* genes. The CKX enzyme activity was measured in the same plant material.

The *TaCKX1* showed the highest cumulative level of expression among ten *TaCKX* genes tested in seedling roots of Polish breeding lines and English mapping population (Quarrie i in. 2005). The relative level among breeding lines ranged from 1 up to 23. The cumulative expression for next two genes: *TaCKX6* and *TaCKX11* was lower in the same lines and mapping population, however was on the same level in Canadian mapping population (Cuthbert i in. 2008). The ranges of their relative levels were narrow. Cumulative level of *TaCKX5* expression in tested lines was lower than for *TaCKX1*, *TaCKX6*, and *TaCKX11* but differed among individual lines from 1 to 50 times.

The *TaCKX1* was the main gene expressed in 7 DAP kernels among ten other tested. The number of copies was on similar level than in seedling roots and relative level of expression for individual lines ranged from 1 to 6. The level of expression for the other *TaCKX* was several dozen to 200 times lower.

The correlation coefficients for the highly expressed *TaCKX1* showed: 1) negative correlation between the gene expression in 7 DAP kernels and grain yield, 2) positive correlation between the gene expression in 7 DAP kernels and plant height, 3) negative correlation between the gene expression in seedling roots and the number of kernels and the yield.

The data on expression profiles of ten *TaCKX* genes in leaves, inflorescences and 14 DAP kernels of selected breeding lines will be completed and presented.

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Selecting for high anthocyanins and high carotenoids to enhance the antioxidant activity of durum wheat

Antonella Pasqualone[a], Barbara Laddomada[b], Isabella Centomani[a], Stefano Di Leone[a], Carmine Summo[a], Giovanni Mita[b], Rosanna Simeone, Agata Gadaleta and **Antonio Blanco[c]**

[a]*Department of Soil, Plant, and Food Sciences, Food Science Section, University of Bari, Italy*
[b]*Institute of Sciences of Food Production, National Research Council, ISPA - CNR, Italy*
[c]*Department of Soil, Plant, and Food Sciences, Plant Breeding Section, University of Bari, Italy*

antonio.blanco@uniba.it

In recent years, the interest towards functional properties of foods has increased progressively and a relevant role has been played by antioxidant compounds, such as anthocyanins, carotenoids and phenolic acids, able to scavenge free radicals. Purple wheat contains higher levels of anthocyanins than conventional wheat cultivars. Durum wheat is also characterized by relevant levels of carotenoids, compared to soft wheat. The aim of this work has been to breed durum wheat lines characterized by high anthocyanin and high carotenoid levels. This strategy enhances the antioxidant activity of the derived whole meal and processed products (Pasqualone et al., 2015). Purple wheat line CItr 14629 (*Triticum turgidum* ssp. *durum* (Desf.) Husnot), derived from an Ethiopic landrace kindly provided by the United States Department of Agriculture (USDA), and the Italian durum wheat cv. Grecale, characterized by high carotenoid content, have been crossed and the segregant generations grown and evaluated according to the pedigree method. Twenty-five F_6 lines has been selected on the basis of pericarp color, plant height, 1000-seed weight, protein content, and yellow index of whole meal flour (known to be related to carotenoid pigments). The total anthocyanins content of these 25 lines, all characterized by maximum score for visual inspection of pericarp color (score= 5, range 1-5 from yellow to dark purple), ranged from 0.94 to 43.06 mg/kg cyanidin-3-glucoside (Cy-3-Glu) on dry matter, whereas the purple parent showed a mean value of 37.13 mg/kg Cy-3-Glu (d.m.). The variability of this range evidenced that the visual evaluation of pericarp color, usually considered for selecting purple wheats (Knievel et al., 2009), is not sufficiently precise compared to spectrophotometric measure of total anthocyanins in the extracts obtained from whole meal. The lines showing the highest anthocyanin content will be characterized for the single anthocyanins and for the major phenolic acids by means of HPLC analysis. No correlation was found between total anthocyanin content and the a^* colorimetric index of whole meal. Protein content of the selected lines was comprised between 13.3% and 17.9% d.m., whereas 1000-seed weight ranged from 33.3 g to 56.3 g. Yellow index was in the range 12.9-14.6. Plant height of the selected lines was lowered to 80-90 cm in comparison to 130 cm of the Ethiopian parental line. The selected lines are currently under agronomic evaluation in different field trials and different nutritional traits will be particularly considered to assess the grain and flour quality.

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Breeding for new climate- screening agro morphological traits of wheat genepool

Sonja Petrović[a], Sunčica Guberac[a], Andrijana Rebekić[a], Tihomir Čupić[b] and Vlado Guberac[a]

[a]*Faculty of Agriculture in Osijek/Department of Plant Production, University of J.J. Strossmayer, Croatia*
[b]*Agricultural Institute Osijek/Department for Breeding and Genetics of Forage Crops, Croatia*

spetrovic@pfos.hr

Wheat's ability to adapt to multiple environments enabled its production on more worldwide land area than any other crop. In recent years, climate change and frequent changes of weather conditions made adaptability of wheat to changeable conditions a highly desirable trait. For that reason, efforts of breeders around the globe are focused on increasing the wheat adaptability and tolerance to drought and heat stress, together with other abiotic stress induced by changeable weather conditions, while preserving yield stability. Application of molecular marker technology to identify adaptable genotypes is widely used and it has its advantages, although in order to get a complete picture of a specific genotype, it has to be tested in the field. Drought tolerance and wheat adaptability are complex traits, so estimation of phenotypic diversity and trait association will always be present in crossing parents as well as in segregating generations. The aim of this research was to screen wheat genotypes based on their agronomical and morphological traits, linked to adaptability to changeable climate conditions, and to single out the best performing genotypes as a material for further testing. The experiment was carried out during vegetation years 2013/2014 and 2014/2015 in Eastern part of Croatia. Phenotyping of a panel of 365 diverse wheat accession originating from different countries was carried out on 19 morphological and 12 agronomic traits. Principal Coordinates Analysis (PCoA) on morphological traits explained total of 33.74% variability among cultivars by first two PC. All cultivars were grouped in 17 clusters. Hierarchical cluster analysis, based on agronomic traits, yielded 10 clusters, while 7 genotypes were separated as single clusters. Based on principal component analysis (PCA), first three principal components explained 62.3% of overall diversity between genotypes. The first PC consisted of no. spikelets, no. grain/ear, no. grains/spikelet, ear mass and grain mass/ear, tkm, hl, moisture and yield were in PC2, while days to heading and plant height in PC3. Days to heading had lowest (CV=3.3%) while ear length had highest variability (CV=18.5%) in the examined panel of wheat accessions. Furthermore, days to heading was positively correlated to plant height (r=0.49**), ear length (r=0.18**) and number of spikelets per ear (0.18**). Significant positive correlation (r=0.70**) between days to heading in climatically very different two vegetation years revealed certain genotypes better adapted to changed environment conditions.

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Identification of allelic variation at Powdery mildew resistance gene Pm3 in a collection of tetraploid wheats

Luciana Piarulli[a], Agata Gadaleta[b], Domenica Nigro[a], Giacomo Mangini[a], Massimo A. Signorile[a], Silvana Zacheo[a], Marina Pasquini[c], Antonio Blanco[a] and **Rosanna Simeone[a]**

[a] *Department of Soil, Plant and Food Sciences - Sect. Genetics and plant breeding, University of Bari Aldo Moro, Italy*
[b] *Department of Agricultural and Environmental Sciences, University of Bari Aldo Moro, Italy.*
[c] *C.R.A. Research Unit for Cereal Qualitative Improvement (QCE), Italy*

rosanna.simeone@uniba.it

Powdery mildew caused by *Blumeria graminis* f. sp. *tritici*, is one of the most important wheat disease occurring world-wide in temperate climates. Race-specific resistances to this pathogen are given by the *Pm* genes. The *Pm3* is the only cloned resistance gene, localized on the short arm of wheat chromosome 1A and existing in several allelic forms (*Pm3a* to *Pm3g, Pm3k* to *Pm3t).* The aim of this work was the screening of a collection of 233 tetraploid wheat genotypes (*Triticum turgidum* L.) in order to identify new *Pm3* functional alleles.
The phenotypic screening was carried out by infecting seedlings with the isolate O2 (virulence/avirulence pattern: *Pm1, Pm2, Pm3c, Pm4a, Pm4b, Pm5, Pm6, Mli / Pm3a, Pm3b, Pm3d).*
Out of 233 tetraploid wheat genotypes tested, 34 accessions (14.6%) were found to be resistant. The genotypic evaluation of the identified resistant lines was performed for the *Pm3* haplotype by the screening for the presence/absence of an STS marker amplifying a 946 bp fragment diagnostic for the presence of *Pm3-like* gene. The genotypes showing the presence of a *Pm3-like* gene, were screened with a set of seven *Pm3* allele primer combinations (*Pm3a* to *Pm3g)* in order to identify the specific allele for each line. Based on virulence/avirulence pattern of O2 isolate used for resistance tests, only *Pm3b* allele was amplified in the resistant lines. In order to identify new *Pm3b* alleles a set of PCR primer combination was designed on Chinese Spring *Pm3b* gene sequences. These primer pairs were used to obtain the complete sequence of *Pm3b* coding region in four resistant lines and in four susceptible, chosen as control. Several differences in terms of SNPs and small indels were found, resulting in new allelic variants of the *Pm3b* gene. Out of eight lines analyzed, the line AG-85 of ssp. *durum*, showed the higher level of polymorphism compared to Chinese Spring *Pm3b* gene sequences.
These results could be of greater importance in the identification of new allelic variants and also could be used in MAS (Marker Assisted Selection) programs.

Reaction of European winter wheat cultivars to six isolates of *Puccinia triticina*

Magdalena Radecka-Janusik, Paweł Cz. Czembor and Piotr Słowacki

Plant Breeding & Acclimatization Institute – National Research Institute, Department of Plant Breeding and Genetics, Radzików, 05-870 Błonie, Poland

m.radecka-janusik@ihar.edu.pl

Leaf rust caused by the biotrophic fungus *Puccinia triticina* Eriks. is one of the most important fungal disease affecting wheat worldwide. Genetic resistance is the most economical, effective and ecologically sustainable method of controlling the disease. Over 70 leaf rust resistance genes have been characterized. Efficient utilization of genetic resistance relies on an appropriate knowledge of the leaf rust resistance genes and of their effectiveness against virulence displayed by population of the pathogen. Resistance genes can be postulated at seedling stage based on well-known gene-for-gene concept. Infection type (IT) pattern (virulent/avirulent phenotype) observed on differential set with known resistance genes is used to infer about *Lr* genes in cultivars after inoculation with the same *P. triticina* isolates. Presented work is a part of larger project aiming at gene postulation using molecular markers and phenotype data. But from reaction of wheat cultivars to inoculation of *P. triticina* isolates we can also select highly resistant and susceptible genotypes providing valuable information for breeders.

NILs of cv. 'Thatcher' (TcNILs) carrying known *Lr* resistance genes and set of 162 European winter wheat cultivars collection including 83 cultivars registered on national list of COBORU (Research Centre for Cultivar Testing, Poland) were tested with six *P. triticina* isolates. After testing 38 TcNILs, it was noted that all isolates were virulent to genes *Lr2c*, *Lr10*, *Lr11*, *Lr14a*, *Lr14b*, *Lr18*, *Lr23*, *Lr24*, *Lr27+Lr31*, *Lr30*, *Lr33*, *Lr44*, *LrB* (Carina) and avirulent to *Lr1*, *Lr9*, *Lr19*, *Lr20*, *Lr25* and *Lr28*. For the rest of TcNILs, at least one isolate displayed different IT than the other isolates. Among wheat cultivars tested, 36 were resistant to all isolates, 36 were resistant to five isolates and 63 were susceptible to all six *P. triticina* isolates. The other 27 wheat cultivars were resistant to 1─4 isolates of the pathogen.

Early generation differences in concentration of trace elements in winter wheat grain

Andrijana Rebekić, **Sonja Marić**, Sonja Petrović, Sunčica Guberac and Zdenko Lončarić

Faculty of Agriculture/Department of Plant Production, University of J.J. Strossmayer, Croatia

smaric@pfos.hr

Based on previous results 10 parental genotypes were selected according to Fe, Zn and Cd grain concentrations. Targeted Zn and Fe grain concentration achieved by biofortification needs to be higher than 40 mg kg^{-1} while Cd concentration in grain has to be lower than 0.2 mg kg^{-1}. In 2013 first filial generation was produced from 19 crossing combinations. The concentration of Fe, Zn and Cd in the solution of grain samples was determined by ICP-OES. Cd grain concentration ranged from 0.032 mg kg^{-1} to 0.0844 mg.kg^{-1}. Fe grain concentration ranged from 32.09 mg kg^{-1} to 47.94 mg kg^{-1}, while Zn grain concentration ranged from 17.78 mg kg^{-1} to 39.81 mg kg^{-1}. Measurement of Fe grain concentration revealed only one combination with higher (2.75%) concentration, compared to parental average. In 10 crossing combinations grain Zn concentration was higher than parental average, ranged from 2.37% to 118%. Regarding Cd grain concentration one crossing combination was singled out having lower concentration than parental average. Based on this results promising F1 crossing combinations were selected and sown for further testing, that will potentially lead to selection towards biofortification for low Cd, and high Fe and Zn grain concentration.

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Development of transcript-based markers tagging 1RS wheat-rye translocations

Yong Weon Seo, Yong Jin Lee and Woo Joo Jung

Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Korea

seoag@korea.ac.kr

Wheat-rye translocations in the form of 1AL.1RS and 1BL.1RS are frequently used in wheat breeding. Short arm of rye chromosome 1 carries a number of resistance genes for insects and fungal diseases and has been reported to enhance yield performance. We developed 1RS specific markers with two different strategies, using public sequence databases and drought stress treated cDNA analysis, respectively. Total 411 ESTs of wheat homoeologous group 1 (1A, 1B and 1D) were downloaded and applied to sequence comparative analysis with other Poaceae family. We designed 142 cross-species primer pairs (that are expected to exist in most Poaceae species) and screened primers using 'Chinese Spring', 'Petkus (rye, 1RS donor of 1BL.1RS translocation)', and near-isolines (NILs) of either presence or absence for 1RS. Consequently, we developed four transcript-based 1RS specific markers, 3 for both 1AL.1RS and 1BL.1RS and the other for 1BL.1RS only. cDNA-AFLP analysis was conducted using cDNAs from PEG treated NILs. Transcript-derived Fragments (TDFs) that showed high similarity to 1AS, 1BS and 1DS were selected and BLASTed against chromosome survey sequences of wheat and rye. Primer pairs specific to rye sequences were designed and confirmed as wheat-rye chromosome specific. These markers could be used in marker-assisted selection during breeding of 1RS translocation lines.

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Development of bread wheat lines with super-soft endosperm texture - the carriers of two genes *Ha* and *Ha-Sp* for grain softness

Alexander V. Simonov [a], Tatyana A. Pshenichnikova [a], Ludmila V. Shchukina [a], Angela K. Chistyakova [a], Alexandra A. Shishkina[b] and Ekaterina D. Badaeva[b]

[a] *Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia (ICG SB RAS)*
[b] *Vavilov Institute of General Genetics Russian Academy of Sciences (VIGG RAS)*

sialexander@bionet.nsc.ru

Endosperm texture parameters of bread wheat are determined by the one gene *Ha* in 5D chromosome. Due to the multiply allelism the grain texture in different cultivars varies from soft and floury to hard and vitreous. The dominant allele determines the formation of floury endosperm and present in soft-grain wheat cultivars. At the same time, manifestation of the trait is environmentally dependent. The cultivars Chinese Spring (CS) is soft-grained with floury grains and during milling gives the flour with small particle size (PS). The spring cultivar Rodina is hard-grained with vitreous grains having PS after milling twice as high than in CS. The winter introgression line 84/98^{w} on the genetic basis of Rodina carries the homoeologous gene *Ha-Sp* from *Aegilops speltoides* Tausch in 5A chromosome. Therefore, the line has a soft semi-vitreous grain and small PS. The aim of the work was to combine these two genes, *Ha* on 5D chromosome and *Ha-Sp* introgressed from 5S into 5A, in one genotype. It was supposed that two genes will make the grain softness more expressed and less dependent from environment. For this purpose, cv. CS was crossed with the line 84/98^{w}. In the number of generations from F_2 to F_8 the selection was made for vitreousness and PS in spring habit plants. From the pedigrees of late generations the super-soft lines with floury endosperm were selected. Low vitreousness of grain and small PS retained both in greenhouse and field conditions. The differential staining of chromosomes was done in order to prove the presence of introgressed fragments of 5S chromosome of *Ae. speltoides* in the genome lines. Physical properties of dough were tested in F_6-F_8 lines on alveograph. The lines have low flour strength. Just that kind of flour is preferable for manufacturing pastries and biscuits without using chemical powders.

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Molecular selection in three winter wheat populations for *Fhb1* resistance gene to Fusarium head blight

Piotr Słowacki, Magdalena Radecka-Janusik and Paweł Cz. Czembor

Plant Breeding & Acclimatization Institute – National Research Institute, Department of Plant Breeding and Genetics, Radzików, Błonie, Poland

p.slowacki@ihar.edu.pl

Marker Assisted Backcrossing (MAB) may be an efficient strategy to increase breeding progress, especially for traits such as resistance to fusarium head blight (FHB) that are difficult to select for under field conditions and that are controlled by multiple genes. The purpose of the presented work is to incorporate resistance gene *Fhb1* located on chromosome 3B into three Polish advanced breeding lines of winter wheat (recurrent parents, RPs): SMH8527 (Smolice Plant Breeeding Company, IHAR Group), DL414/10 (Danko Plant Breeding Company) and STH1178 (Strzelce Plant Breeding Company, IHAR Group). The donor of the resistance gene is wheat line AIII72 (F_5BC_2) derived from the cross between Sumai 3 and Polish cultivar Muszelka. This line was confirmed with molecular markers to contain *Fhb1* gene.

In order to reduce the size of the donor chromosome segment containing the target locus, plant selection in the offspring populations (F_1BC_1) is focused on selecting individuals with the target gene (*Fhb1*) and recombination events between the target locus and linked flanking markers (recombinant selection). DNA polymorphism between RPs and *Fhb1* gene donor at ten SSR flanking markers (gwm389, barc238, barc12, gpw7080, gwm493, barc131, wmc754, gpw3248, barc92 and cfp1274) spanning ca 40cM, allowed us to choose two polymorphic flanking markers and two central markers (confirming the presence of *Fhb1* gene).

A total of 360 samples (120 for each combination) were tested with two flanking markers (cfp1274 and gwm389) and one of the two central markers (UMN10 or cfb6033). Thirty one individuals were chosen after the analysis (SMH8527 – 8 ; DL414/10 – 8 ; STH1178 – 15) for next backcross.

Genetic structure of NS wheat core collection

Dragana Trkulja[a], Ankica Kondic-Spika[a], Ljiljana Brbaklic[b], Borislav Kobiljski[b], Sanja Mikic[a], and Gordana Surlan-Momirovic[c]

[a] *Institute of Field and Vegetable Crops, Serbia*
[b] *Biogranum, Serbia*
[c] *Faculty of Agriculture, University of Belgrade, Serbia*

dragana.trkulja@nsseme.com

Association analysis proved to be a useful method for detection of quantitative trait loci for agronomic important traits in wheat. It relies on linkage disequilibrium and involves the use of unstructured populations that are both genotypically and phenotypically characterized to detect statistically significant associations between genetic polymorphism and trait variation. Therefore, to avoid declaring false positive marker-trait associations, it is essential to determine population structure of analyzed material. The aim of this study was to assess the presence of population structure within the genetic material chosen for association mapping of yield and yield related traits in wheat.

The wheat Genetic Collection of the Institute of Field and Vegetable Crops, Novi Sad, Serbia is comprised of 1000 cultivars originating from 38 countries worldwide. During a five year period, a number of agronomic important traits was evaluated to select desirable parents for creating offspring with high yield potential in our agro-climate region. Within this collection, a set of 282 cultivars from 26 countries was chosen according to their performances for 11 agronomical important traits (heading, flowering, stem height, spike length, number of spikelets per spike, number of sterile spikelets per spike, number of grains per spike, spike weight, grain weight, thousand grain weight and grain yield). The accessions were further genotyped with 31 (out of initial 40) microsatellite markers distributed along all three wheat genomes. Population structure of analyzed material was assessed by the software program STRUCTURE 2.3.4. Apart from that, all subpopulations derived from structure analysis were evaluated by the principal coordinate analysis (PCoA).

Structure analysis revealed presence of three subpopulations, designated as Q1, Q2 and Q3. The largest number of accessions, 127, belonged to subpopulation Q2. The subpopulation Q1 counted for 101, while Q3 included 54 accessions. The genotypes were grouped according to their origin as well as pedigree data. Therefore, the most of cultivars created in Serbia, were assembled in Q1, as well as the most of Russian and Romanian genotypes. The USA accessions, were predominantly grouped in Q2, together with a half of Australian and almost all Croatian and Hungarian cultivars. Finally, the subpopulation Q3 was consisted of accessions from Western Europe, the UK, France and Germany. Analysis of pedigree data indicated that in each subpopulations, a few cultivars dominated in family tree. Thus, Bezostaya 1 and accessions from breeding program of Nazareno Strampelli dominated in Q1. Within the pedigre of Q2, the most frequent was cultivar Marquis, as well as cultivars derived from Norin-10/Brevor cross, while in Q3 the most present were Vilmorin-27, Hybride De Joncquois, Viking and Squarehead. The PCoA showed almost clear separations of subpopulations and certain overlaps within groups could be a result of the frequent use of particular genotypes in different breeding programs worldwide.

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Domains of products of *Rht* genes in bread wheat

Andrii Venger, Olga Kolesnyk and Anastasiia Honcharova

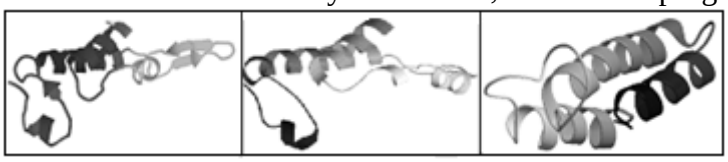
Department of General and Molecular Genetics, The Plant Breeding and Genetics Institute – National Center of Seed and Cultivar Investigation, Ukraine

venger87@ukr.net

Dwarfing genes are classified according to their sensitivity to externally applied gibberellins (GAs). The introduction of the reduced height (Rht)-B1b and Rht-D1b semidwarfing genes led to impressive increases in wheat (*Triticum aestivum* L.) yields during the Green Revolution. The reduction in stem elongation in varieties containing these alleles is caused by a limited response to the phytohormone GA, resulting in improved resistance to stem lodging and yield benefits through an increase in grain number. GAs are key plant hormones, which determine various aspects of growth and development such as stem elongation, seed germination, leaf expansion, flower development etc. In wheat and other plants the biosynthesis of gibberellic acids is determined by products of *rht* genes. According to partial analysis of products of those genes in wheat's there were domains of DELLA proteins detected. DELLA protein is a key negative regulator of gibberellin signaling. Although how DELLA regulates downstream gene expression remains unclear, DELLA has been proposed to function as a transcriptional activator (Yoshida et. al., 2014).

In our work we researched *rht*-gene products of *T. aestivum* using alignment with different amino acids sequences in psi-blast program of National Centre of Biotechnological Information. As results there were DELLA and GRAS protein domains identified.

DELLA protein models (Fig.1) were submitted by SWISS-MODEL (Swiss Institute of Bioinformatics) which is a fully automated protein structure homology-modelling server, accessible via the ExPASy web server, or from the program DeepView (Swiss Pdb-Viewer).



A B C

Fig. 1. DELLA protein GAI models (A, B) and hypothetical cytosolic protein (C)

Proteins in the GRAS family are known as major players in gibberellin signaling, which regulates various aspects of plant growth and development. Mutation of the SCARECROW (SCR) gene results in a radial pattern defect, loss of a ground tissue layer, in the root. The PAT1 protein is involved in phytochrome A signal transduction. A sequence, structure and evolutionary analysis showed that the GRAS family emerged in bacteria and belongs to the Rossmann-fold, AdoMET (SAM)-dependent methyltransferase superfamily. All bacterial, and a subset of plant GRAS proteins, are predicted to be active and function as small-molecule methylases. Several plant GRAS proteins lack one or more AdoMet (SAM)-binding residues while preserving their substrate-binding residues (Zhang et. al., 2012). Although GRAS proteins are implicated to function as transcriptional factors, the above analysis suggests that they instead might either modify or bind small molecules.

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Zhang, D., Iyer, L.M., Aravind, L., 2012. Bacterial GRAS domain proteins throw new light on gibberellic acid response mechanisms. Bioinformatics 28, 2407–2411.

Breeding for improved gluten strength in winter durum wheat

Gyula Vida, Ottó Veisz, and Mariann Rakszegi

Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Hungary

vida.gyula@agrar.mta.hu

Durum wheat has a number of traits which make it ideal for pasta-making. Due to its high yellow pigment content, attractive products can be manufactured without the addition of eggs. It also has high protein content, while its strong gluten matrix retains the starch molecules during cooking, with the result that the surface of the pasta does not become slimy and sticky and the pasta keeps its shape. Gluten strength can be determined using several techniques. The determination of the gluten index, proved to be a useful technique for use in durum wheat breeding programmes. The gluten index is a stable, highly heritable genetic trait, in different experiments the heritability ranged from 0.84 to 0.95. Winter durum wheat represents a special group of genotypes within the durum wheat species, and its cultivation is restricted primarily to countries in Eastern and Central Europe. Testing of the first genuine winter durum wheat lines began in Martonvásár in 1982. These originated from the Odessa breeding programme and had excellent cold tolerance, but their technological quality was poorer than that of spring durum wheat varieties. Improving cold tolerance and technological quality simultaneously is no easy matter. The varieties used as sources of cold tolerance did not have satisfactory gluten strength, so high quality facultative durum wheat varieties were also included in the crossing programme. The present poster reports on the results achieved in improving the gluten strength of winter durum wheat during the last 15 years.

An analysis was made on the data of experiments on registered winter durum wheat varieties, advanced lines and the most promising lines in the breeding programme to determine the effect of introducing tests on the gluten index during the period 2000–2015. The number of lines included in the analysis ranged from 15 (2000) to 31 (2008–2015). Due to the nature of the experiment, the lines included in the analysis differed from year to year, so the data were compared with those of a check variety (‘Mv Makaróni’) sown every year.

Improvements in the gluten index had to be achieved while maintaining or improving the values of other traits (cold tolerance, protein content, yellow index). The results achieved between 2000 and 2015 were compared with that of ‘Mv Makaróni’, registered in 2001. When ‘Mv Makaróni’ was developed, priority was given to yield stability, giving special attention to improving cold tolerance. An excellent level of cold tolerance was successfully combined in this variety with high protein and yellow pigment content, but in most years its gluten strength did not satisfy the criteria raised by the processing industry. After introduction of gluten index measurement, a period of ten years was required before having over 70% of new breeding lines with a gluten index significantly better than that of ‘Mv Makaróni’. Since 2007 this proportion has been stable at over 80%. The lines examined included a high proportion of genotypes with good-to-excellent gluten strength (until 2007: 5–64%, since 2008: 40–87%), so this trait no longer represents a bottleneck in the selection of advanced lines. Using selection based on this parameter, the gluten strength of winter durum wheat lines can be improved sufficiently to make them competitive with high quality spring varieties.

The demand for high quality durum wheat is increasing worldwide, while the production of this crop species is in decline for various environmental and economic reasons. This contradiction could be resolved partially by expanding the production area of winter durum wheat varieties with good pasta-making quality.

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Agronomic performance of two generations (F_{12} and F_{13}) of thirteen winter wheat composite cross wheat populations with differing cultivation histories in 2014/15

Odette Weedon[a], Sarah Brumlop[a], Sven Heinrich[a], Andreas Boening[a], Maren Elsner[a], **Maria Renate Finckh[a]**, Edith Lammerts van Bueren[b,c], Edwin Nuijten[c], Monika M. Messmer[d], Jörg Peter Baresel[e], Isabelle Goldringer[f], Mikó Péter[g], Mária Megyeri[g], Anders Borgen[h], Søren Rasmussen[i], Bruce Pearce[j] and Martin Wolfe[j]

a. *Department of Ecological Plant Protection, University of Kassel, Germany*
b. *Wageningen UR Plant Breeding, Wageningen University, the Netherlands*
c. *Louis Bolk Institute, the Netherlands*
d. *Research Institute of Organic Agriculture (FiBL), Switzerland*
e. *Chair of Plant Nutrition, Technical University of München, Germany*
f. *Quantitative Genetics and Evolution – Le Moulon, INRA, France*
g. *Centre for Agricultural Research, Hungarian Academy of Sciences, Hungary*
h. *Agrologica, Denmark*
i. *Department of Plant and Environmental Sciences, University of Copenhagen, Denmark*
j. *The Organic Research Centre, Elm Farm, United Kingdom*

mfinckh@uni-kassel.de

As environmental and agronomic conditions are heterogeneous between and within locations, diversity within varieties or crop populations should increase adaptability to the changing and variable range of growing environments. The additional pressure of plant genetic diversity loss has driven novel breeding approaches such as composite cross populations (CCPs) and other genotype mixtures, thereby increasing both intra- and inter-varietal diversity and ensuring a "wider adaptation" capacity for crop varieties (Döring *et al*., 2011). A winter wheat (*Triticum aestivum* L.) CCP was created by intercrossing 20 varieties in 2001, in collaboration with the Elm Farm Research Centre and the John Innes Institute. In 2005, a seed batch of the F_4 was equally divided and distributed to Hungary and Germany. In 2007, it was decided to submit one of the CCPs to changes in environment every year. A pattern was developed between eight partners whereby these "cycling" populations would be grown in a plot of >100m^2 and sent to the next cycling partner the following year. The aim of the project was to compare a total of 13 populations that all originated from the same seed batch in 2005, but that have been exposed to vastly different climatic conditions over time at one site (Germany). In 2014/15, the second experimental year, saved seed from 2013 (F_{12}) and harvested seed from 2014 (F_{13}) were sown in order to compare two generations in one growing season. The experimental year 2014/15 was characterized by long dry periods, particularly between February and June 2015, and under these dry conditions most populations out-yielded the selected reference varieties. There was no effect of differential seed size on the two generations for most agronomic characteristics for each population. Although the harvested TGW of both the F_{12} and the F_{13} of each population was not significantly different from one another, there were still significant differences of harvested TGW between the populations in the F_{12}. These significant differences of harvested TGW were no longer present in the F_{13} between each population after one year under the same management system. These results indicate that the heritability of seed size is low, as has been shown before (Silvertown, 1989) and that seed size variation tends to be a result of phenotypic plasticity, which is thought to be adaptive, especially as the result of environmental variation (Marshall *et al*., 1985; Vaughton and Ramsey, 1998; Lehtilä and Ehrlén, 2005).

Yield stability analysis for three winter wheat composite cross populations under organic and conventional management over five years

Odette Weedon[a], Sarah Brumlop[a], Sven Heinrich[a] and **Maria Renate Finckh[a]**

[a.]*Department of Ecological Plant Protection, University of Kassel, Germany*

mfinckh@uni-kassel.de

Increasingly uncertain climatic conditions threaten the security and stability of agricultural systems (Østergård *et al*., 2009; Döring *et al*., 2011) and a better understanding of genotype x environment (GxE) interactions is needed to shift the agricultural focus from manipulating environments to grow crops to creating crops that fit into the environment (Østergård *et al*., 2009; Lammerts van Bueren *et al*., 2012). GxE interactions play a pivotal role in assessing yield stability of crops and the challenge of changing climatic conditions necessitates that new crop cultivars should have broad adaptability, stable agronomic performance over a range of environments and management systems, and generally high yields (Akcura *et al*., 2006). The high genetic diversity (Hi-D) approach is relevant to organic and low-input agricultural systems due to the comparatively large environmental variability found in these systems, where increased genetic diversity is better able to cope with higher biotic and abiotic stresses (Annicchiarico and Filippi, 2007; Döring *et al*., 2010; Dawson and Goldringer, 2012). Three winter wheat composite cross populations (CCP) have been grown under both organic and conventional conditions since the F_4 in the research fields of the University of Kassel in Neu Eichenberg, Germany. A number of yield stability indicators were calculated for the three CC populations (YQ, Q and Y) grown under both organic and conventional conditions over five harvest years (2008, 2010, 2012, 2014 and 2015). The reference varieties Achat and Capo were also grown alongside the organic CCPs for comparison. In terms of yield stability at the organic site, the CCP YQ showed the highest stability for three values in the analysis (R^2, MSE and W^2), making it the most stable population in terms of the stability analysis of the populations and varieties under organic management. Low values for CV% and environmental variance (S^2), being the variance of the genotype yields over all environments, indicate that the Q population was the most stable for these two stability measures. The reference varieties Achat and Capo had a general tendency towards less stability than the organic CC populations, only for the value of S^2 did Capo show some stability in terms of environmental variance over the organic CC populations YQ and Y. The conventional populations did not show many of the same tendencies in terms of population stability as found in the organic system. The conventional Y population had a slope of 1.24 and a R^2 value of 0.99. This population also had the lowest MSE of all the populations (0.007), indicating a higher degree of stability for these measures. The gradient of the slope was much steeper in comparison to the organic Y population, which had a slope closer to 1, indicating that under conventional management, the Y population was able to react more strongly under favourable conditions. The conventional YQ population had the highest mean yield over the five years and the lowest value for W^2, indicating the greatest stability in terms of GxE interactions. In comparison, the organic YQ population had a higher degree of stability with the best values for more stability measures, indicating that perhaps under less favourable conditions, the organic YQ population is more stable and able to adapt to challenging conditions. The CCP Q population under both organic and conventional management showed a high degree of yield stability, indicating that this population, although not the highest yielding, may be the best suited for a wider range of conditions.

Selection of Leaf Rust-resistant haploid and diploid genotypes

Weigt D.[1], Kiel A.[1], Nawracała J.[1], Tomkowiak A.[1], Kurasiak-Popowska D[1]., Karpińska M.[1], Ługowska B.[2], Niemann J[1]., Mikołajczyk S.[1]

[1]*Department of Genetics and Plant Breeding, Poznan University of Life Sciences, Poland*
[2]*DANKO Plant Breeding Ltd.*

Biotechnology techniques, such as in vitro culture or molecular marker, are useful tools which can shorten the process of development of new crop varieties. The application of new technologies makes the wheat breeding strategy more effective and enables the development of varieties with a high yield potential, resistance and tolerance to stress. In vitro haploid production increased the precision and efficiency of selection by obtaining completely homozygous wheat lines in a single generation. The selection of genotypes for further breeding can be assisted by molecular markers to identify important traits. This process can take place at an early stage of plant development. Moreover, molecular testing of haploids can be carried out in a laboratory immediately after receiving the plants.

The aim of the study was to select wheat leaf rust-resistant haploid and diploid genotypes by using *Xwmc221* and *GB* markers of the *Lr 19* gene.

Haploids and double haploids were obtained from an F_4 generation of plants by crossing two genotypes with the *Lr19* gene (T36, T39) and two cultivars: Hondia and Ozon. Haploidy was achieved by androgenesis in the anther culture according to the method developed by Weigt et al. (2012). Ploidy was determined by flow cytometry. Two markers: *Xwmc221* (Gupta et al., 2006) and *GB* (Prins et al., 2001) were used to identify the *Lr19* gene. The microsatellite marker *Xwmc221* exhibited a codominant pattern, amplifying a 200bp fragment characterising resistant genotypes and a 220bp fragment corresponding to susceptible genotypes. The *Gb* STS marker amplified a 130bp fragment only from resistant genotypes.

The molecular analysis of the F_4 generation plants showed small differences between the two systems of markers. *Xwmc 221* and *GB* markers, characteristic of the resistant gene, were identified in 37 and 34 plants of 66 genotypes tested, respectively. Most of the F_4 generation plants turned out to be heterozygous. The haploids obtained from the plants in which the marker of the *L19* gene was identified were used for molecular analysis. 58% of them gave PCR products characteristic of resistant genotypes. In the rest of the haploid plants obtained from the susceptible genotypes no markers of the *Lr19* gene were found. There were no differences in the presence of these markers between the hapLoids and DH_1 lines.

DNA markers were used to select the haploids. The DH lines were more effective than the selection of the F_4 generations, in which some plants remained heterozygous.

The research was carried out as part of project NCBR-PBS2/A8/25/2013 implemented by the BIOTRIGEN consortium.

Early selection of winter wheat (*Triticum aestivum* L.) recombinants resistant to eyespot using enzymatic and molecular markers for *Pch1* gene in haploid and double haploid (DH) lines

Halina Wiśniewska[a], Michał Kwiatek[a], Maciej Majka[a], Jolanta Belter[a], Magdalena Gawłowska[a], Tadeusz Adamski[a], Tadeusz Drzazga[b] and Bogusława Ługowska[c]

[a] *Institute of Plant Genetics of the Polish Academy of Sciences, Poland*
[b] *Malopolska Plant Breeding Company Ltd.*
[c] *Danko Plant Breeding Company Ltd.*

hwis@igr.poznan.pl

The *Pch1* gene has been incorporated into cultivated wheat from *Aegilops ventricosa* to provide an effective resistance to eyespot caused by the necrotrophic fungi *Oculimacula acuformis* and *O. yallundae*. The aim of this study was to shorten the selection of wheat recombinants bearing the *Pch1* gene. We investigated 98 breeding lines of winter wheat obtained by crossing of two resistant forms of wheat carrying the *Pch1* gene (KBH 4942/05, KBP0916) with two breeding lines (SMH 8592, POB 32408) carrying technological quality traits and the Polish cultivar Jantarka. The analysis of molecular (*Xust2001-7DL*) and enzymatic (endopeptidase *Ep1*) markers allowed to select 22 plants with the *Pch1* gene. These forms were crossed with maize (*Zea mays* L.) to get haploids and then double haploids by colchicine treatment. 512 haploids were obtained and analyzed to identify the eyespot resistance gene. 473 haploids with the *Pch1* gene were treated with colchicine to get 439 double haploids lines (DH). The presence of the *Pch1* gene was also examined in double haploids using the same endopeptidase and SSR markers. Finally, DH lines were tested for resistance to eyespot in field experiments by spraying with a conidial-mycelium suspension of *Oculimacula acuformis* and *O. yallundae*. Our study demonstrated the possibility of early selection of recombinant lines resistant to eyespot using haploid forms.

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Identification of eyespot resistance genes in breeding lines of hexaploid wheat (*Triticum aestivum* L.)

Halina Wiśniewska[a], **Michał Kwiatek[a]**, Maciej Majka[a], Magdalena Gawłowska[a], Marek Korbas[b], Jakub Danielewicz[b] and Jolanta Belter[a]

[a]*Institute of Plant Genetics, Polish Academy of Science, Poznań, Poland*
[b] *Institute of Plant Protection, National Research Institute, Poznań, Poland*

mkwi@igr.poznan.pl

Eyespot is an important fungal stem-base disease of wheat (*Triticum aestivum* L.) and other cereals in temperate regions. It is caused by two necrotrophic fungi *Oculimacula yallundae* and *O. acuformis*, which usually appear simultaneously on the field. First symptoms can be observed during the autumn pullulating. Eye-shaped, elliptical lesions on the lower portion of the stem can weaken stem bases and cause them to bend or break. Crops from infected plants are shorter with reduced quality and yield losses of up to 50%. There are three eyespot resistance genes (*Pch1, Pch2* and a QTL on chromosome 5A), which have been characterized in hybrid forms of wheat. *Pch1* is the most potent of these and was introduced into wheat from the wild grass *Aegilops ventricosa*. However, the *Pch1* locus is associated with lower yield in the absence of the disease, which limits its use for breeding elite varieties. On the other side, both *Pch2* and *Pch-QTL-5A* originate from the French wheat cultivar Cappelle Desprez, but confer only a moderate level of resistance and are unlikely to prevent yield loss under high disease pressure. The aim of this study is to search a breeding collection of hexaploid wheat for eyespot resistance. We chose enzymatic and SSR (Single Sequence Repeats) markers to track the resistance genes. The molecular analyses were verified by inoculation tests during the seedling and stem elongation stages. Additionally, natural infection by *O. yallundae* and *O. acuformis* was observed in four locations in Poland. Moreover, the weather conditions were monitored. Finally, the yield components were calculated and compared with marker results in order to evaluate the influence of the genotype on phenotype values of breeding lines.

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Microsporogenesis and drought tolerance of wheat

Yuliya Yanushevska[a], Jolanta Groszyk[a], Anna Nadolska-Orczyk[b] and **Waclaw Orczyk[a]**

[a] *Department of Genetic Engineering, Plant Breeding and Acclimatization Institute – National Research Institute, Poland,*
[b] *Department of Functional Genomics, Plant Breeding and Acclimatization Institute – National Research Institute, Poland*

w.orczyk@ihar.edu.pl

Plant male reproductive development is very sensitive to environmental factors. Even mild stresses applied during early phases of floret development irreversibly affect microsporogenesis and lead to pollen abortion. In self-pollinating and cleistogamic crops such as wheat and barley this significantly reduces grain number per spike and yield.
The goal of the project was to find molecular markers for tolerance to this particular stress in wheat, i.e. proper grain filling of the spike in plants subjected to drought in the sensitive phase of anther development.
In order to identify specific stages of anther development, steps in microsporogenesis were correlated with auricle distance (AD) in the two wheat cultivars Fundulea and Sava. Anthers in stages between meiosis and young microspores were found in plants with an AD of 5cm. As this represents the most sensitive stage of microsporogenesis, it was selected for the start of the drought treatment.
Plants of tested cultivars were grown in the greenhouse and watered to 60-70% of field capacity (FC). Drought stress of 20-30% FC, was applied for 5 days starting from the preselected stage (AD = 5cm) of plant growth. Out of more than 110 wheat genotypes derived from a wide range of climatic conditions, 36 genotypes were selected based on their region of origin and general characteristics of drought response for detailed analysis. Pollen viability, grain number per spike and anther-specific gene expression in normal and drought stress conditions were characterized. Pollen viability of certain cultivars was significantly reduced in drought stressed plants. The percentage of viable pollen after drought ranged from 14% to 98% when compared to plants grown in control conditions. The number of grains per spike in drought stressed plants was up to 39% lower when compared to controls. Lower viability of pollen was correlated with lower number of grains per spike.
Anther-specific expression of several genes was analyzed in control and drought-stressed plants. Expression patterns of *TaInv3* (apoplastic cell wall invertase3) and *TaDIS1* (ortholog of *OsDIS1* in rice) significantly differed between drought sensitive and drought tolerant genotypes. Expression patterns of other tested genes (*TaInv5* and *TaABA-8'OH*) were similar in tolerant and sensitive cultivars in both types of growth conditions. The possible function of the genes in drought response will be discussed.

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Parallel flash and poster presentations: Other cereals

Dynamic management of winter barley genetic resources

Lothar Frese[a], Marion Nachtigall[a], **Lorenz Bülow[a]**, Frank Ordon[b] and Jens Léon[c]

[a] *Institute for Breeding Research on Agricultural Crops, Julius Kühn-Institut, Germany*
[b] *Institute for Resistance Research and Stress Tolerance, Julius Kühn-Institut, Germany*
[c] *INRES Plantbreeding, Universität Bonn, Germany*

lorenz.buelow@jki.bund.de

Genebanks apply static *ex situ* management systems to conserve plant genetic resources for food and agriculture. By doing so, genebanks facilitate users' access to germplasm and related data. While genebanks maintain accessions, i. e. a spatial and temporal part of the evolutionary process, dynamic management strategies aim at promoting the continued adaptation of crops to changing environmental conditions (Bretting and Duvick, 1997). An effective and efficient approach for a dynamic management system with wheat was described by Goldringer et al. (2001) and is being developed by the INRA at Le Moulon (France) towards an on-farm management system involving farmers. The transfer of this approach to barley was recommended by the German Federal Ministry of Food and Agricultural in its expert program for plant genetic resources and the Julius Kühn-Institut was requested to develop an institutional network for the dynamic management of winter barley genetic resources.

From a total of 227 German winter barley varieties released between 1914 and 2003, a set of 58 varieties was genetically analyzed using SSR markers. Among these, 32 genotypes representing the genetic diversity of the whole set were selected to produce a highly recombinant winter barley population. In the years 2008 to 2015, the 32 selected winter barley varieties were crossed according to the Multi-parent Advanced Generation Inter-Cross (MAGIC, Cavanagh *et al*., 2008) scheme resulting in a set of lines with each line being a descendent of all 32 initial varieties and thus harboring parts of all 32 initial genomes. Aliquot amounts of seeds from 324 of these lines were combined in 2015 to form a highly heterozygous population and grown for multiplication.

Starting in 2016, sub-populations of this material will continuously be grown under high and low input conditions at different locations within Germany. In order to promote the development of differently adapted germplasm, 10 ecogeographically contrasting locations within Germany were selected. Adaptation of winter barley sub-populations to different climatic, soil and agricultural input conditions will be monitored for at least 6 years. Based on samples taken in each year, changes in the allele frequencies within and between locations will be monitored at the DNA level. In parallel, an information system will be developed for consistent documentation of varieties and lines, crossing schemes, composition of (sub)populations, cultivation conditions, characterization and evaluation data, and for subsequent data analysis.

As a long-term result, an institutional network for the dynamic management of winter barley genetic resources will be realized comprised of populations and sub-populations cultivated at different locations and exhibiting a wide genetic variation within and between populations, adapted to regional agricultural conditions, and with potential for future adaptation to climate changes. The network will be supported by a consistent documentation. All plant genetic resources developed by the network will be publicly available according to the rules of the Multilateral System of the International Treaty.

The current project has in parts been funded by the Association for the Promotion of Private Plant Breeding in Germany (GFP) and by the German Federal Ministry of Education and Research (BMBF).

Landraces and obsolete cultivars of common oat – valuable and unused genetic resources

Maja Boczkowska[a] and Agnieszka Onyśk[b]

[a] *Plant Breeding and Acclimatization Institute – National Research Institute, National Centre for Plant Genetic Resources, Poland*
[b] *Plant Breeding and Acclimatization Institute – National Research Institute, Department of Functional Genomics*

m.boczkowska@ihar.edu.pl

Landraces and old, obsolete cultivars are a rich and still under-utilized source of diversity that could be easily introduced into breeding programs. They are characterized by yield stability, broad adaptation, tolerance to diseases and a greater competitiveness in the presence of weeds.

The study was performed to answer the following questions: 1) How broad is the genetic pool of Polish oat germplasm?; 2) What proportion of the diversity is attributable to individuals of landraces, obsolete and modern cultivars?; 3) Is there any population structure?; 4) Are the gene pools of different types of germplasm distinct? The answers to these questions might be helpful during the evaluation of primitive and obsolete germplasm for oat breeding programs in Central Europe.

Inter Simple Sequence Repeats (ISSR) were used to study the genetic diversity of 12 modern Polish cultivars, 23 old Polish cultivars, 19 native landraces and five contemporary European cultivars. Each of the tested accessions was represented by 24 individuals, which were analysed separately.

The results of ISSR analysis of Polish cultivars unambiguously indicated that currently grown cultivars share a very similar genetic background, although they derive from three different breeding companies and their gene pool is significantly distinct from old cultivars. This is due to the non-utilization of old resources in Polish breeding programs. The landraces have the highest external and internal diversity and also contain alleles that were lost during breeding in the twentieth century. Knowledge about the level of differentiation within accessions can be practically applied to the control of the proper maintenance and management of gene-bank collections. Summing up the results of this and previous studies leads to the general conclusion that a significant part of the gene pool of landraces was irreversibly lost long before the start of the ex-situ conservation programs. Another point is that the set of ISSR markers used here can successfully identify old Polish cultivars that were erroneously classified as landraces and can rule out contamination of the landraces by modern cultivars seeds. However, the primer set does not allow all landrace accessions to be identified. Due to their significant internal differentiation and the overlap between variation patterns, identifying molecular markers to characterize individual populations might be impossible. This study tries to remind breeders about forgotten and unused gene pools stored in gene banks.

WHEALBI: Wheat and barley legacy for breeding improvement: an EU project to link genomics and agronomy

Gilles Charmet[a], Nils Stein[b], Joanne Russel[c], Robbie Waugh[c], Luigi Cattivelli[d], Fred Van Eeuwijk[e], Beat Keller[f], Jacob Lage[g], Nathan Morris[h], Sébastien Crépieux[i], Emmanuelle Lagendijck[j] and Ronan Pendu[j]

[a] *INRA-Université Clermont II, UMR GDEC, Clermont-Ferrand, France*
[b] *Leibniz – Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK) Gatersleben, Germany*
[c] *The James Hutton Institute (JHI), Dundee, Scotland, United Kingdom*
[d] *Consiglio per la ricerca e sperimentazione in agricoltura (CREA) Fiorenzuola d'Arda PC, Italy*
[e] *Wageningen University, The Netherlands*
[f] *Universität Zurich, Zurich, Switzerland*
[g] *KWS-UK, Thriplow near Cambridge, United Kingdom*
[h] *National Institute of Agricultural Botany (NIAB), Cambridge, United Kingdom*
[i] *ARCADIA International, Brussels, Belgium*
[j] *INRA-transfert SA, Paris, France*

gilles.charmet@clermont.inra.fr

WHEALBI is granted 5 M€ by EU-FP7 (Grant no 613 556) for 5 years starting January, 2014. It involves 18 partners (8 academics, 7 industry /SME) in 9 countries and aims at improving European wheat and barley production in competitive and sustainable cropping systems. Germplasm will be selected and characterised by next-generation-sequencing. Adaptive traits will be evaluated in both transnational field experiments and precision phenotyping platforms. Germplasm will be stored in a bio-repository and associated data in knowledge bases that will represent a valuable legacy to the community. Whole genome association scans will be conducted for several traits, signatures of adaptive selection will be explored, and allele mining of candidate genes will reveal new variation associated with specific phenotypes. Pre-breeding tools will be developed to optimize the efficiency of allele transfer from unadapted germplasm into elite breeding lines. New methodologies will explore how to optimally exploit the large amount of new genotypic and phenotypic data available. Ideotypes with improved yield stability and tolerance to biotic and climatic stresses will be evaluated in innovative cropping systems, particularly organic farming and no-till agriculture, and an economic evaluation will be conducted. Results will be disseminated to a broad user community, highlighting the benefits and issues associated with the adoption of sustainable wheat and barley crop production.

In 2015, WHEALBI has produced significant achievements in several ways. First of all, exome sequence of 512 barley and 512 wheat accessions covering the range of genetic diversity have been produced. These raw data are currently being processed (cleaning, quality control, SNP calling) to be released to WHEALBI partners in early 2016. As an evidence of the value of these data, we already received demands from several consortia to have access, which will be effective soon after first exploitation within WHEALBI. These 1024 accessions have also been planted in the field, at 7 locations for each species, spanning over Europe from Scotland to Turkey. This will allow a comprehensive study of adaptation to a wide range of climatic conditions, and exome data will give insights into its genetic components.

In 2016, exome polymorphism will also be "mined" to explore the genetic bases of key adaptive traits, and used in statistical models to improve genetic dissection breeding efficiency, as illustrated by the 1000 bulls genome project (http://www.1000bullgenomes.com). A smaller collection of diverse barley and wheat varieties will also be studied in innovative, more sustainable cropping systems, including organic, to anticipate the needs of future European Agriculture.

Variation in leaf traits in spring barley (*Hordeum vulgare* L.)

Anna Florence[a], Steve Hoad[a], Richard Ennos[b] and Peter Hoebe[a]

[a] *Crop and Soils Research, SRUC, United Kingdom*
[b] *Institute of Evolutionary Biology, University of Edinburgh, United Kingdom*

Anna.Florence@sruc.ac.uk

Cultivated barley originated in the Fertile Crescent and is one of the founding crops of modern agriculture. Having spread throughout the continent of Europe it is now widely grown for animal feed and the malting industry. Today only a limited number of modern barley cultivars are widely grown which contain limited genetic diversity. These have been intensively selectively bred to maximise yield under high input conditions, increase harvest index (HI) and improve crop uniformity. Before the introduction of these modern cultivars European barley comprised of a collection of landraces, each of which was genetically diverse and adapted to local environmental and management conditions. There is increasing interest in landraces as sources of useful genetic variation which could be re-introduced to boost resource-use-efficiency (RUE) in traits such as photosynthetic efficiency, especially under environmental stresses in the context of climate change.

In order to do this the existing diversity within and between European barley landraces will need to be studied in more detail. Previous studies have shown that landraces from Northern Europe have become adapted to near continuous summer daylight by losing their daylight responsiveness requirement for flowering. Could there be other morphological and physiological adaptations to optimise light capture and photosynthesis affecting characters such as leaf shape and chlorophyll content to varying local environmental conditions? This would allow Northern European landraces to exploit the longer hours of sunlight and Southern European landraces to cope with drought and high temperature stress during their limited growing seasons.

This study assesses the diversity present in European landraces spanning a wide latitudinal and longitudinal range in traits associated with light interception efficiency and conversion efficiency. Morphological and physiological measures, which feed into the overarching measures of interception and conversion efficiency, such as leaf area, leaf chlorophyll content, photosynthetic rate and leaf angle were recorded under common garden field conditions in two successive years to study the range of variation in these landraces. A high level of variation within and between landraces was reported before and after flowering in interception and conversion efficiency traits compared to modern cultivars. Canopy angle and chlorophyll content which are involved in the interception efficiency showed strong links with the latitude of origin. The landraces from northern latitudes have a planophile canopy structure with the canopy becoming more erectophile as you head south through Europe. The leaf chlorophyll content also decreased as latitude decreases.

The future of this research will help advise climate smart breeding for improved light RUE to maximise yields under changing conditions. Looking at the traits related to photosynthetic efficiency at a morphological and physiological level and understanding how they are adapted to local conditions can help advise on possible useful sources of germplasm.

Different cold sensitivities in rice unvailed by anther morphologies and genome-wide expressions

Yuji Kishima[a], Kei Ogasawara[a], Seiya Ishiguro[a], Hu Na[a], Kaien Fujino[a] and Yutaka Sato[b]

[a] *Research Faculty of Agriculture, Hokkaido University, Japan*
[b] *NARO Hokkaido Agricultural Research Center, Japan*

kishima@abs.agr.hokudai.ac.jp

Rice plant at booting stage exposed to low temperature resulted in pollen sterility, while varying degrees of the impaired pollen sterilities were observed among the cultivars examined. Based on difference of the decreasing pollen fertility, we attempted to find correlation between pollen sterility and morphological abnormality in anther structure. Each cultivar showed different feature in terms of the anther morphology and proportion. The various structural changes of the anthers were largely divided into two abnormalities related to tapetum and locule structures. Tapetum hypertrophy was detected as one of the abnormal structures in the anthers examined, although this was not typical symptom for the pollen sterility. The degree of pollen abortion after the cool treatment was positively correlated with the morphological abnormalities in locule structure and anther length. Our observation demonstrated that tapetum hypertrophy in rice is unlikely to become a versatile indication of pollen sterility due to cold stress at booting stage. We also found the cold sensitive cultivars causing pollen sterility without the abnormal anther structures. Genome-wide transcriptome analyses sorted out the two types of the cold sensitive cultivars, in which the cold-treatment enhanced or impaired overall expressions. The cold-sensitive cultivars showing the anther structural abnormality enhanced the genome-wide expression after the cold-treatment, while the other cold-sensitive cultivars without anther structural changes indicated a decline of the overall expressions. Our results provided new indications for the cold-sensitive cultivars causing the pollen sterility due to cold stress at the booting stage.

Genomic prediction in a Finnish breeding programme of six-row barley

Timo Knürr[a], Mika Isolahti[b], Outi Manninen[b], Ismo Strandén[a], Esa Teperi[b], Merja Veteläinen[b] and Esa A. Mäntysaari[a]

[a] *Natural Resources Institute Finland (Luke), Green technology/Biometrical genetics, Finland*
[b] *Boreal Plant Breeding Ltd, Finland*

Timo.Knurr@luke.fi

Genomic selection has the potential to accelerate genetic gain and to reduce phenotyping costs in commercial breeding programmes. In this study, we evaluated accuracy of genomic prediction in field trial data of a commercial breeding programme of six-row barley.
The data comprised 1934 doubled-haploid and single-seed-descent barley lines representing 333 crosses. Genotype information was available for 5821 SNPs. For validation, the data was split into a training population with 1124 lines and two validation populations with 429 and 381 lines, respectively. To imitate a practical selection scenario in a real breeding programme, the two validation populations were chosen from young crosses mainly derived from lines in the training population. Phenotypes were measured in multiple environments across Finland in several years.
We compared the performance of two genomic evaluation models (multi-trait GBLUP and single-trait BayesB) and multi-trait BLUP with pedigree-based relationship matrix but no genomic information. Accuracy of genomic estimated breeding values (GEBV) and estimated breeding values (EBV) in validation lines was assessed by the correlation coefficient between GEBV/EBV and averaged phenotypic observations corrected for trial-specific effects. The validation correlations were calculated across lines from different crosses (r_{ac}) for genomic models and for pedigree-based BLUP. Correlations across lines within crosses (r_{wc}) were assessed only for the genomic models, as EBVs from pedigree-based BLUP are identical for lines from the same cross.
We evaluated the barley lines for total yield, time to ripening and grain protein content. Total yield was subdivided into three traits according to three different growing environments based on zone and soil. Heritabilities were between 0.30 and 0.45 for the three total yield traits, 0.57 for time to ripening and 0.64 for protein content.
For the three total yield traits, validation accuracies across lines in different crosses (r_{ac}) were on average 0.31 for GBLUP, 0.29 for BayesB and 0.17 for pedigree-based BLUP. As expected, within-cross r_{wc} was lower, with values of 0.26 for GBLUP and 0.19 for BayesB, respectively.
Accuracies of GEBV for time to ripening were considerably higher than for the three total yield traits. Here, r_{ac} was 0.52 for GBLUP and 0.57 for Bayes B. EBV coming from pedigree-based BLUP had r_{ac} of only 0.21. Within-cross r_{wc} was 0.39 for GBLUP and 0.43 for BayesB.
Likewise, accuracies for protein content were higher than for total yield traits. In validation across different crosses, r_{ac} was 0.62 for GBLUP, 0.67 for BayesB and 0.48 for pedigree-based BLUP. Within-cross r_{wc} was 0.42 for GBLUP and 0.49 for BayesB.
The results indicate that genomic models predict future phenotypes better than pedigree-based BLUP. GBLUP performed slightly better than BayesB for total yield traits, whereas the opposite was found for the traits with higher reliabilities (protein content and time to ripening). As expected, there was some loss in accuracies when the genomic breeding values were used to predict performance within crosses.

Analysis of the genetic and environmental factors influencing grain quality in oats

Pilar Martinez Martin, Catherine Howarth, Irene Griffiths and Sandy Cowan

Institute of Biological Environmental & Rural Sciences (IBERS), Gogerddan, Aberystwyth University, Wales, UK

mjm7@aber.ac.uk

Grain quality of oats is important to meet the requirements of the milling industry and to enhance the value of the crop for the grower. Developing oat varieties with high milling quality is constrained by a lack of detailed information on how genetic differences and environmental and management conditions impact on grain quality. Focussing on key milling quality characters, i.e. specific weight, kernel content, hullability and thousand grain weight, four winter oat varieties (Gerald, Mascani, Tardis and Balado) were grown under conventional and organic regimes at six geographical locations in 2012-13 and 2013-14. In addition, grain yields and oil, protein and β-glucan content of the groat was determined. The length, width, area, roundness and weight of the grain and groat, were measured using non-destructive methods. The influence of environment, management conditions and genetic differences on grain quality parameters, were determined by statistical analysis. The results were statistically significant for area, length and width between varieties and locations (p-value <0.05). These parameters also showed correlation with kernel content, hullability and thousand grain weight. Further investigations will examine the effect of nitrogen fertilisation on milling quality traits as well as using a mapping population to determine their genetic basis. These results obtained will be used to develop new varieties for the milling industry, by the farmer to assess quality on farm prior to marketing, and by the plant breeder in selection programs, where genetic improvements in milling quality may be made more precisely and rapidly than previously.

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Genotype and environmental impact on spring cereal yield and quality

Vija Strazdina [1], Arta Kronberga [1], Aina Kokare [1], Mauritz Åssveen[2] and **Ievina Sturite**[2]

[1] *Latvian University of Agriculture, Institute of Agricultural Resources and Economics, Latvia*
[2] *Norwegian Institute of Bioeconomy Research, Food Production and Society, Norway*

ievina.sturite@nibio.no

Longer and warmer growing seasons in the northern part of Europe may widen utilization of spring cereal genetic resources. Moreover, this may lead to more diverse cereal use in the human diet. In recent years, hull-less barley (*Hordeum vulgare* L.) and hull-less oat (*Avena sativa* L.) have been included in food production in Europe due to their diverse health benefits. It is well documented that hull-less barley and oat grains contain insoluble and soluble fractions of dietary fibre and other bioactive compounds that makes beneficial effects on human health. Compared with hulled grain, hull-less grain contains less fibre, more protein and lipids, and has higher energy value. In Latvia, new hull-less varieties have been developed in order to ensure both high productivity and increased grain quality. In Norway, such breeding programs have been limited but interest in growing hull-less genotypes has increased. The main objective of this study was to evaluate hull-less barley and oat yield and grain quality grown under different climatic conditions in Latvia and Norway.

Field trials were established in Latvia and Norway in spring 2015. The Latvian trial locations were at Priekuli (57.32°N) and in Stende (57.10°N) while the Norwegian trial locations were at Apelsvoll (60,7°N) and at Kvithamar (63,5°N). Two Latvian hull-less barley varieties (cv Irbe and cv Kornelija) and one Norwegian hull-less barley line (GN 03386) were tested along with one Latvian hulled barley variety (cv Rubiola) and one Norwegian hulled barley variety (cv Tyra) under conventional management system. Similarly, one Latvian and two Norwegian hull-less oat varieties (cv Emilija, cv Bikini and cv Nudist, respectively) along with one Latvian and one Norwegian hulled oat variety (cv Laima and cv Odal, respectively). The variety trials were performed as block trials with four replicates. Application of fertilizers and chemical pesticides were according to agronomical practice in each country. The grain yield was recorded and grain quality such as thousand-kernel weight (TGW), test weight (HL-weight) were determined. Crude protein and fat concentration was measured.

Preliminary results indicate that hull-less varieties have significantly lower yield potential than hulled varieties regardless of cereal species and species origin ($P<0.001$). In Latvia, the origin of hull-less barley and oat varieties had no effect on grain yield. In Norway, the Norwegian hull-less genotypes produced significantly greater grain yields than the Latvian hull-less genotypes ($P<0.05$). Thus, environmental impact was more clearly pronounced at locations in the orth than in the south. The TGW for the hull-less barley variety cv Kornelija was significantly larger than for hulled and other hull-less barley varieties at both locations in Norway and at Stende in Latvia ($P<0.001$). At all locations, hull-less oat varieties had significantly lower TGW than hulled oat varieties ($P<0.01$). For the hull-less oat variety cv Emilija the crude protein concentration ranged from 15 to 16.3% and it was significantly higher than for the hull-less oat varieties cv Nudist and cv Bikini regardless of trial location ($P<0.001$). High protein content was measured in grains at Kvithamar suggesting that light conditions during the growing season might stimulate protein accumulation. Hull-less oat grains also had significantly higher fat concentrations than hulled oat grains at all locations ($P<0.001$). Consequently, the better quality of hull-less spring cereals over hulled spring cereals confirm that there is a need for breeding programs that improve genotype yield potential.

Genetics of malting barley 'processability'

Pete Hedley[a], Luke Ramsay[a], Joanne Russell[a], Stuart Swanston[a], Kelly Houston[a], Jenny Morris[a], Hazel Bull[a], Richard Keith[a], Chris Warden[b], Ian Slaiding[a] and **William Thomas**[a]

[a] *James Hutton Institute, Invergowrie, Dundee, UK*
[b] *Campden BRI, Coopers Hill Road, Nutfield, Surrey, UK*

Bill.Thomas@hutton.ac.uk

A high level of hot water (malt) extract is just one of the attributes of a 'good' malting barley cultivar. Almost as important is the 'processability' of a malting lot in a brewhouse and/or distillery. Poor processors tend to cause problems in lautering and downstream in brewing and distilling, resulting in operational inefficiency. Processability is poorly defined as a character but problems generally occur due to poor modification in a lot, either due to an excess of cell wall compounds and/or a lack of cell wall degrading enzyme activity.

We grew sets of 100 spring and winter barley genotypes in trials for harvest 2013 and 2014 under malting and feed barley fertilizer regimes. Cleaned and graded seed from each plot was analysed for grain nitrogen, beta-glucan, and total pentosan content. Each year, taches of low and high nitrogen content grain from each genotype were micro-malted and analysed for extract and a range of parameters that reflect aspects of filtration. Residual malt was sent to Campden BRI for small-scale mash filtration (Vmax) testing. Data were analysed to derive estimated means for each genotype in each year x environment combination for all variates.

Genotypic data for each line was combined with the phenotypic data in Genome Wide Association Studies (GWAS) to identify Quantitative Trait Loci (QTL) that affected the malting quality parameters. Means from all sites were analysed together to detect QTL that were either consistent across the four environments or interacted with the environment. Two spring and three winter barley cultivars were selected as good processors and two and three respectively as bad. Developing grain was harvested from each at DGS71 and DGS75 from trials grown under malting and feed regimes and RNA extracted for gene expression analyses. The locations of up and down regulated transcripts were compared to the QTL locations to help refine potential candidate genes affecting processability.

The feed regime generally produced the expected mean change in malting quality parameters, although there were some interesting exceptions. Importantly, some genotypes were less affected by the change in grain nitrogen content and these were also more stable for processability characteristics such as wort viscosity and Vmax. Genetic analyses revealed a number of chromosomal segments affecting processability characteristics but none were associated with the known major loci affecting beta-glucan synthesis and breakdown. The lines that we studied were largely monomorphic in the regions where such loci are located, suggesting that they have been fixed in elite UK malting barley germplasm. We did, however, detect variation in the region of arabinoxylan synthesizing and degrading genes, suggesting that selection for reduced arabinoxylan content and/or increased degradation would improve processability. Supporting evidence for this hypothesis came from significant changes in expression of a transcript affecting arabinoxylan synthesis that was located in the region of several QTL affecting processability characteristics.

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Virulence of *Puccinia triticina* on triticale in Poland

Grzegorz Czajowski and Paweł Cz. Czembor

Department of Plant Breeding and Genetics, Plant Breeding and Acclimatization Institute - National Research Institute at Radzików, 05-870 Błonie, Poland

g.czajowski@ihar.edu.pl

Leaf rust, caused by the biotrophic fungus *Puccinia triticina* Eriks., is one of the most important fungal disease affecting triticale worldwide. This disease can cause serious epidemics and yield losses. One hundred sixty isolates of *Puccinia triticina* were collected in the years 2014 – 2015 from triticale in four locations in Poland. These isolates were analyzed for virulence variation on thirty-five near isogenic Thatcher NILs with known *Lr* resistance genes.

Populations of the pathogen collected in 2014 revealed high virulence frequency, ranged from 60 to 100%, toward the majority of *Lr* genes: *Lr2c, Lr10, Lr11, Lr13, Lr14a, Lr14b, Lr18, Lr21, Lr29, Lr30, Lr33* and *Lr44*. The frequencies of virulence to lines with genes *Lr1, Lr2a, Lr3, Lr3bg, Lr16, Lr17, Lr23, Lr26, Lr36, Lr38 and LrW* were low. No virulence was found to resistance genes *Lr9, Lr19, Lr25*. These resistance genes were the most effective. Pathotypes of *P. triticina* were identified with the use of 15 NILs possessing resistance *genes Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr9, Lr11, Lr15, Lr17, Lr19, Lr21, Lr23, Lr24, Lr26* and *Lr28.* Thirty seven pathotypes from eighty isolates of *P. triticina* were distinguished.

Virulence tests for populations collected in the year 2015 are under way and the results will be presented during the conference.

Resistance to Powdery mildew in winter barley in Poland

Jerzy H. Czembor[a] and Aleksandra Pietrusińska[a]

[a] *Plant Breeding and Acclimatization Institute-NRI / National Centre for Plant Genetic Resources, Poland*

j.h.czembor@ihar.edu.pl

Winter barley is an important cereal crop and it is grown in Central and Western Poland. The powdery mildew caused by *Blumeria graminis* f. sp. *hordei* is one of the most frequently observed diseases on winter barley in Poland and can cause considerable yield losses. The use of resistant cultivars is an effective method to control powdery mildew and the incorporation of new genes for resistance to powdery mildew into barley cultivars has been very useful in controlling powdery mildew. Mlo resistance has become a very important source of powdery mildew resistance in barley because there is no known virulence for these genes.

The present investigation describes the introduction of the *mlo* gene for resistance to powdery mildew (*B. graminis* f.sp. *hordei*) into winter barley cultivars characterized by high and stable yield potential under Polish conditions. We aimed at field testing of the obtained lines with Mlo resistance for their agricultural value.

Four cultivars (Souleyka, Titus, SU Vireni and Metaxa) as high yielding parents were used. In addition, existing resistance genes to powdery mildew in these cultivars were preserved. Two lines (BKH 735 and line 42) as parents with Mlo resistance were used. Line BKH 735 was obtained in the Laboratory of Applied Genetics PBAI-NRI Radzików in 2002-2011. Selection for presence of the *mlo* gene was conducted in backcross populations by phenotyping in the field (natural infection) and under greenhouse conditions (differential barley lines for resistance genes for powdery mildew and differential fungus isolates). In addition, to confirm the presence of the *mlo* gene in backcross populations MAS strategy was applied using SSR markers HVmlo1 and HVmlo3.

Field trials with 200 F_4BC_1 lines were conducted during 2015/16 in 3 locations: in Central (Radzików) and Western Poland (Szelejewo, Wiatrowo). The parental lines were used as control. The aim of these trials was to obtain information on agricultural value of obtained lines. Our results demonstrate the practical use of the introduction of Mlo resistance into background of winter barley germplasm with valuable economical characteristics in Polish agricultural conditions.

This work was conducted in the project: Interaction between powdery mildew (*Blumeria graminis* f.sp. *hordei*) resistance determined by *mlo* gene and economical value characteristics in winter barley. 2014-2020. Programme: Basic Research for Biological Progress in Crop Production; Funded by the Ministry of Agriculture and Rural Development Proj. No. 4-1-04-3-01 (27).

BSMV-VIGS as a post-transcriptional gene silencing strategy to dissect biological function of *ScBx1* in rye

Jolanta Groszyk[a], Mariusz Kowalczyk[b], Yuliya Yanushevska[a], Anna Stochmal[b], Monika Rakoczy-Trojanowska[c] and Wacław Orczyk[a]

[a] *Department of Genetic Engineering, Plant Breeding and Acclimatization Institute – National Research Institute, Poland*
[b] *Department of Biochemistry and Crop Quality, Institute of Soil Science and Plant Cultivation State Research Institute, Poland*
[c] *Department of Plant Genetics, Breeding and Biotechnology, Warsaw University of Life Sciences, Poland*

j.groszyk@ihar.edu.pl

Benzoxazinoids (BX) are a group of defense related molecules accumulated as glycosylated precursors in grasses and sporadically in few dicot species. They are considered as important components of defense against *Ostrinia nubilatis* (European corn borer) and *Diabrotica virgifera* (Western corn rootworm) in maize. The compounds were also found to be involved in plant allelopatic interactions and stress tolerance. DIBOA (2,4-dihydroxy-1,4-benzoxazin-3-one), the most important BX synthesized in rye and the final product of benzoxazinoid biosynthetic pathway, is catalyzed by five enzymes encoded by *Bx1÷Bx5* genes. The first step of BX synthesis pathway is catalyzed by indole-3-glycerol phosphate lyase. Proteins with this enzyme activity are encoded by at least three genes *Bx1*, *TSA* and *Igl*, all showing high sequence similarity. The recently identified gene was designated as a putative rye ortholog of *Bx1* based on high similarity (considering nucleotide and expected amino acid sequence as well as genomic intron-exon structure) to wheat *TaBx1*. The goal of the project was to verify experimentally biological function of the isolated rye gene. The *Barley stripe mosaic virus*-induced gene silencing (BSMV-VIGS) system was selected as an experimental tool for functional analysis of this gene. Selected cDNA fragment of the gene CDS was cloned into plasmids carrying β and γ subunits of BSMV-based vectors. The resultant plasmids with *ScBx1* fragment were used as the templates for *in vitro* transcription. The mixture of BSMV:α, BSMV:β*ScBx1* and BSMV:γ*PDS* transcripts (BSMV:*ScBx1* silencing vector) and the mixture of BSMV:α, BSMV:β(-) and BSMV:γ(-) (BSMV:00, control vector) were used for inoculation of rye seedlings. Leaves with symptoms of BSMV infection were collected 14 and 21 days post inoculation (dpi) and used for expression analysis of *ScBx1* and quantification of BXs. The relative expression of *ScBx1* in control plants (BSMV:00) in leaves collected 14 and 21 was assumed as 1.00 and served as a reference to compare expression in experimental plants. Gene expression in plants treated with BSMV silencing vector (BSMV:*ScBx1*) were lowered to the level of 1.5% (14 dpi) and 24.5% (21 dpi) of the expression found in the control. BX concentration in leaves of control plants collected 14 and 21 dpi ranged from 989.69 to 3474.74 and from 829.54 to 2292.90 µg/g of dry weight respectively. The amount of BXs in leaves of experimental plants (BSMV:*ScBx1*) collected 14 dpi ranged from 175.45 to 1169.79 and 21 dpi ranged from 152.40 to 929.15 µg/g of dry weight. The results prove that the amount of BXs in plants with silenced *ScBx1* expression was significantly lower. The expression of the analyzed gene including both developmentally-dependent regulation and VIGS-induced silencing were highly correlated with concentrations of BXs. The cumulative results confirm that the analyzed gene is the rye ortholog of *Bx1* and can be functionally annotated as the *ScBx1*. The results also confirm that the VIGS-BSMV system can be used as an efficient tool for functional analysis of rye genes.

Genetic evaluation and colocation of QTLs in seedling stage of F8 rice RILs under drought, salinity and cold stresses

Mahnaz Katouzi[a], Saeid Navvab pour[a], Ahad Yamchi[a], Sayyedeh Sanaz Ramazanpour[a] and Hossein Sabouri[b]

[a] *Department of Plant Breeding, Gorgan University of Agriculture Science and Natural Resource, Iran*
[b] *Department of Plant Production, University of Gonbad Kavous, Iran*

katouzi.mah@gmail.com

Rice is one of the important crops in the world. Abiotic stresses such as salinity, drought and cold are restricting factors in rice production. In order to provide a linkage map of Sepidroud × Anbarbou crosses, an experiment was conducted using 96 inbreed lines and 123 microsatellites, 261 AFLP and 35 ISSR markers. This research was carried out at the University of Gonbad Kavous in 2013-2014. The linkage map covered 1709.29 cM of the rice genome. 96 inbred lines were planted under hydroponic conditions for mapping of morphological traits under salinity, drought and cold stress. Shoot and root weight, biomass, genetic score, leaf area, shoot and root length, root thickness were recorded. Five QTLs mapped for genetic score under drought condition on 3, 5, 6, 7, 10 chromosomes. A colocation of QTLs was reported in the ISSR28-9-E090-M140-1 interval on chromosome 3 for root weight and biomass (*qDRW-3* and *qDBM-3*). In salinity stress, *qSSES-7* and *qSSES-10* had the largest effect on the genetic score with LOD=2.988 and 3.247, respectively and explained 27% of total phenotypic variation. Six QTLs mapped under cold stress for recorded traits where *qCLA-5* had the highest effect on the genetic score with LOD=3.206 and explained 14.3% of total phenotypic variation. These QTLs with high general contribution and tightly linked QTL regions after QTL validation in an independent data set may be useful for marker-assisted selection in drought, salinity and cold tolerance in rice.

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Association analysis of root and shoot traits in rice (*Oryza sativa* L.) using AFLP markers under field drought stress

Mahnaz Katouzi[a], Hossein Sabouri[b], Abbas Biabani[b], Ahmad Reza Dadras[c], Sharefeh Mohammad Alegh[b], Mohammad Javad Behrozbeh[b], Galdi Mohammad Bahlakeh[b] and Reza Karim Koshteh[b]

[a] *Department of Plant Breeding, Gorgan University of Agriculture Science and Natural Resource, Iran*
[b] *Department of Plant Production, University of Gonbad Kavous, Iran*
[c] *Department of Plant Breeding, University of Guilan, Iran*

katouzi.mah@gmail.com

Drought stress is a serious limiting factor to rice production and yield stability in rainfed areas. Knowledge in the area of genetic diversity could aid to provide useful information in the selection of materials for breeding such as hybridization programs and quantitative trait loci mapping. Association analysis was performed using 192 rice genotypes (landrace, introduced and improved) and AFLP markers (35 primer combinations) for 16 traits including number and length of roots, number of roots less than 5 cm, number of roots between 6-7 cm, 8-20 cm, 21-30 cm, the number roots more of than 30 cm, plant height, panicle number, panicle length, shoot and root dry weight, root volume, biomass, straw weight, main panicle length, number of grains per panicle in main panicle, number of primary spikelets. Five statistical models based on GLM and MLM procedures were used by means of TASSEL software (models: Phenotype+ AFLP, Phenotype+ AFLP+Q, Phenotype+ AFLP+PC, Phenotype+ AFLP+K and Phenotype+ AFLP+K+Q). The results showed markers E-*AGT*_M-*AGA*-1, E-*AGT*_M-*AGT*-7, E-*ATC*_M-*AAC*-9, E-*ATC*_M-*AGA*-4, E-*ATC*_M-*AGT*-8 ،E-*ATT*_M-*AAC*-6 and E-*ATT*_M-*AGT*-1 to be associated with total length of root, and explained 40.9, 42.1, 46.2, 45.4, 40.3, 41.6, 43.7% of phenotypic variation, respectively. The markers E-*AGT*_M-*AAC*-3, E-*AGT*_M-*AGA*-19 and E-*AGT*_M-*AGT*-7 were found to be associated with stem weight and coefficients of determination were 51.6, 50.8 and 50.5% respectively. According to the results, nine markers were determined that could be considered to be the most interesting candidates for further studies.

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Identification of pollen fertility restoration markers in rye with CMS Pampa

Kamil Koc[a], Agnieszka Niedziela[a], Barbara Kozber[b], Waldemar Brukwiński[b], Marek Zając[c] and **Piotr T. Bednarek[a]**

[a] *Plant Breeding and Acclimatization Institute – National Research Institute, Radzikow, Poland*
[b] *DANKO Plant Breeders Ltd., Choryn, Poland*
[c] *Poznań Plant Breeders Ltd., Tulce, Poland*

p.bednarek@ihar.edu.pl

The cytoplasmic male sterility (CMS) phenomenon in plants is based on incompatibility of nuclear and cytoplasmic genomes and results in the lack of production of functional pollen. Its implementation into breeding systems of cereals led to the development of hybrids of commercial importance. A good example is an exploitation of heterosis in hybrid rye with CMS-Pampa (Geiger, Schnell 1970). The evaluation of new hybrids in rye requires efficient parental lines that can restore pollen fertility. Pollen fertility is expressed by numerous genes (Miedaner, Glass et al. 2000), and those located on the chromosome 4R (and 1R) explain most of the phenotypic variance of the trait. For marker-based breeding purposes, markers towards these genes are required.

RIL4 mapping population (maintainer (N) x restorer line with CMS-P) was genotyped with DArTseq and DArT markers. A genetic map based on DArTseq and DArT markers was constructed under MultiPoint software (http://www.multiqtl.com). Pollen fertility restoration of the RILs was verified via crossing the maternal plant (cms-P line) with each RIL using visual scale (Geiger, Morgenstern 1975). Composite interval mapping was performed in WinQTL Cartographer whereas association mapping in TASSEL (Bradbury et al. 2007).

The genetic map consisted of 7 linkage groups corresponding to 7 rye chromosomes covering 962 cM. 528 DArTseq and 43 DArT markers were mapped with a few gaps spanning over 27 cM. Composite interval mapping allowed for the identification of a QTL (spanning over 30 cM) with LOD function maximum equal to 30.8 within 1R (covered nearly 150 cM). The marker closest to the QTL was 1.9 cM from its maximum. Association mapping allowed the identification of 292 markers that passed Bonferroni test with the R^2 ranging from 0.12 to 0.56. Some of the associated markers mapped to the 1R chromosome and fell within the pollen fertility restoration QTL. Based on segregation data some of the DArTseqs formed 14 redundant groups located within the QTL. One of the groups, the closest (~2 cM) to the QTL maximum, consisted of 1 skeleton and two redundant markers with R^2 equal to 0.56. The markers could be used for MAS purposes.

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MultiPoint UltraDense software (http://www.multiqtl.com)

Introgression of the *LTP2* gene through marker assisted backcross breeding in barley (*Hordeum vulgare* L.) with *LTP2* gene analysis expression

Anetta Kuczyńska, Karolina Krystkowiak, Krzysztof Mikołajczak, Piotr Ogrodowicz, Maria Surma, Tadeusz Adamski, Michał Kempa, Aneta Sawikowska and Paweł Krajewski

Institute of the Plant Genetics of the Polish Academy of Sciences, Poznań, Poland

akuc@igr.poznan.pl

Among the numerous genes affecting the plant height in barley the semi-dwarfing *sdw1/denso* gene is one of the most important and it has been incorporated to many cultivars. Considering the *sdw1/denso* pleiotropic effects it may be a candidate for further analysis and manipulation in barley breeding. Due to the escalation of the abiotic stresses (e.g. drought, salinity, soil acidity) breeding programs targeting the development of semi-dwarf cultivars are insufficient. That is why new barley germplasm should be supplemented by genes that facilitate growth and development under stress conditions. Backcross breeding enhanced by marker-assisted selection is already a powerful method allows to transfer one or a few genes controlling a specific trait. In our study the integrated approach of combining phenotypic selection with marker assisted backcross breeding for introgression of the *LTP2* gene, in the background of semi-dwarf cultivar, was employed. This study discusses the efficiency of molecular marker application in backcrossing targeting the selected gene. Due to its role in lipid transfer the *LTP2* may be crucial in lipidome modification in response to abiotic stress. Plant material for the study originates from the collection of Department of Biotechnology, Institute of Plant Genetics, PAS and consists of spring barley lines BC_6 with prostrate and erect growth habit, obtained by backcrossing doubled haploid (DH) lines derived from Maresi × Pomo cross combination. These barley varieties were chosen for the sake of large phenotypic diversity. Maresi is a two-rowed, hulled and brewing cultivar which possesses the semi-dwarfing *sdw1*/*denso* gene from Diamant, an X-ray mutant of the variety Valticky, being in the pedigree of cv. Maresi, whereas Pomo is a six-rowed, hulled and fodder cultivar. DH line MPS37 characterized by erect growth habit was chosen as a donor parent and DH line MPS106 – defined by prostrate stature at the juvenile stage – was selected as a recipient parent. In our experiment we aimed to develop an appropriate approach for the BC strategy betterment supported by using molecular tools (enhanced by marker selection – MAB). We employed single nucleotide polymorphisms (SNP) due to their abundance in a genome and adequacy for analysis on a wide range of scales. The SNP marker, that was used as a selection criteria in BC generation production process, was mapped to the gene MLOC_53422 annotated by Ensembl Plants as *LTP2*. The search for stress-induced genes led to the characterization of genes encoding LTP proteins. The *LTP2* gene transcript levels were also observed using Real Time PCR in preliminary experiment. A set of LTP-like proteins was induced in salt or drought stress in barley. Therefore, we assume that the association of this MLOC_53422 gene with the stress response and protein related to the lipid transport mechanism might be the key finding in breeding of stress resistance crops with the use of BC process.

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Wild Barley (*Hordeum vulgare* ssp. *spontaneum*) as a Potential Source of Drought Tolerance Genes for Barley Improvement

Mohammad Mahdi Majidi[a], Mohammad Barati[a], Aghafakhr Mirlohi[a]

[a] *Department of Agronomy and Plant Breeding, Isfahan University of Technology, Iran*

majidi@cc.iut.ac.ir

Drought stress is the main limiting factor for crops production in arid regions, including Central Iran with 120 mm precipitation. As wild barley (*Hordeum vulgare* ssp. *spontaneum*) and cultivated barley (*H. vulgare* ssp. *vulgare*) are cross-compatible, novel variation revealed in the wild form is useful for barley breeders. In the first study a geographically wide germplasm of wild barley, most of them native to Iran, were evaluated for drought resistance. Germination and seedling characters, root morphology and structure, plus field performance of the genotypes were evaluated under different drought stressed environments for two years. A considerable genetic diversity was observed within populations of wild barley, as well as between some elite barley cultivars and the wild barley genotypes. Wild genotypes with suitable characters and high performance under drought stress environments were detected. Most of these genotypes have been originated from Iran, highlighting the importance of this germplasm in barley breeding programs, which has been neglected in previous studies. The results of root evaluations at reproductive stage were in high agreement with performance and drought tolerance in the field. The genotypes with greater root characters, with some exceptions, had relatively better tolerance to drought stress. In the second study from the wild barley collection, 21 with higher diversity were selected and crossed with an Iranian elite barley cultivar "Reihan 03". The BC1F1 from the 21 crosses and BC2F1 from one of the crosses were obtained and grain yield and some agro-morphological traits (plant height, fertile tiller number, biological yield and harvest index) were evaluated. The results of single plant analysis revealed an extremely high diversity within as well as between the crosses. The progenies with the trait mean values out of the two parental ranges were identified for most of the crosses. The plants with un-desirable characters (seed shattering) were eliminated and finally 440 BC1F2 lines and 100 BC2F2 lines were generated. Each of these lines has a part of wild barley genome in the cultivated barley background. Unique combination of barley-wild barley genomes possessing traits of agronomic-importance including drought stress tolerance is expected in the generated lines.

Phenotyping Fusarium head blight resistance of oat by analysis of morphological and biochemical properties of grains

Charlotte Martin[a], Torsten Schirdewahn[b], Romina Morisoli[a], Susanne Vogelgsang[b], Mario Bertossa[a], Brigitte Mauch-Mani[c] and Fabio Mascher[a].

[a]*Agroscope, Institute for Plant Production Sciences, Changins/Cadenazzo, Switzerland.*
[b]*Agroscope, Institute for Sustainability Sciences, Zurich, Switzerland.*
[c]*University of Neuchâtel, Institute of Biology, Neuchâtel, Switzerland.*

charlotte.martin@agroscope.admin.ch

Oats can provide elevated quantities of health promoting compounds, such as β-glucans, and become an important component of a healthy diet. Yet, several reports reveal that oat grains are often found contaminated with different mycotoxins of the group of trichothecenes such as deoxynivalenol (DON) and T2/HT2 toxins. The contamination results from cryptic infections caused by various *Fusarium* species that are mostly invisible on the panicle and on the grains. The present study aims at investigating modifications of yield components and biological properties of the grains caused by these infections. For this, 9 modern oat varieties and 6 Swiss landraces, obtained from the Vavilov Institute, were sown at 3 different sites with and without artificial inoculations with the DON producing strain of *Fusarium graminearum* FG13. Inoculations were done twice at flowering (BBCH 65-69), in the late afternoon with concomitant overhead irrigation. No symptoms appeared on the artificially infected plants in the field. After harvest, grains of the infected and non-infected plots were examined for thousand kernel weight (TKW), weight ratio of grains compared to their hulls, protein content (using NIRS) and β -glucan content. First analyses showed that β -glucan content increases with the infection to different extents between varieties and in all environments. Yet, this is so far the only grain characteristic significantly affected by the infection. The role of β -glucan in fungal infections in cereal is not clear. We suppose that the increase in β -glucan is part of a defense reaction by the plant. The next steps include an analysis of DON content and a study of the ability of β -glucan to absorb the mycotoxin. This study is a part of a Swiss National Research Project "Healthy and Safe Cereals", in which previous work revealed a significant correlation between DON accumulation and β -glucan content in barley kernels.

Impact of Fusarium infections on ß- glucans in barley grains

Charlotte Martin[a], Torsten Schirdewahn[b], Romina Morisoli[a], Susanne Vogelgsang[b], Mario Bertossa[a], Brigitte Mauch-Mani[c] and Fabio Mascher[a].

[a]*Agroscope, Institute for Plant Production Sciences, Changins/Cadenazzo, Switzerland.*
[b]*Agroscope, Institute for Sustainability Sciences, Zurich, Switzerland.*
[c]*University of Neuchâtel, Institute of Biology, Neuchâtel, Switzerland.*

Charlotte.martin@agroscope.admin.ch

Barley grains can provide elevated quantities of ß- glucans, a soluble fibre recognized to provide benefits for human health. Barley products containing enhanced contents of ß- glucans are now receiving an increasing interest from consumers. Barley plants are also hosts for *Fusarium* pathogens, causing Fusarium head blight and accumulating mycotoxins in grains. As these *Fusarium* pathogens affect properties of the grains, this study aims at investigating modifications of ß- glucan content in grains in case of infections. For that, six winter barley varieties were artificially infected in field trials with a DON producing strains of *Fusarium graminearum*, in three sites across Switzerland. Success of infection was controlled by Fusarium Head Blight symptoms on spikes. After harvest, Thousand Kernel Weight (TKW) were compared between infected and non-infected grains to evaluate changes in morphological properties of grains due to the infections. DON accumulation was measured as well as ß- glucan content in both infected and non-infected grains. Our results indicate that ß- glucan content decreased with infection and this in all varieties. The decrease was correlated with the loss of TKW in infected samples and stronger in susceptible varieties. Surprisingly, in varieties with elevated ß-glucan content, we detected lower concentrations of the mycotoxin DON. To further study a possible role of ß- glucan in DON accumulation, additional barley varieties with a broad range of ß- glucan content will be tested in the same experimental display.

Relationship between photoperiodic reaction and susceptibility to Fusarium head blight in spring barley

Krzysztof Mikołajczak, Anetta Kuczyńska, Karolina Krystkowiak, Piotr Ogrodowicz, Maria Surma, Tadeusz Adamski, Michał Kempa, Halina Wiśniewska and Paweł Krajewski

Institute of the Plant Genetics of the Polish Academy of Sciences, Poznań, Poland

kmik@igr.poznan.pl

Fusarium head blight (FHB) is a devastating disease in small grain cereals worldwide. The disease results in the reduction of grain yield and affects its quality. Spikes infection occurs during the flowering and after flowering time mycotoxins accumulated in grain are harmful to both human and animals. It has been reported that the response to pathogen infection may be associated with morphological and developmental characteristics of the host plant, e.g. the earliness, plant height. FHB disease severity is usually evaluated by visual scoring of disease symptoms on spikes (type I and II) and visual scoring of infected kernels (FDK – resistance type III) in harvested samples. Also, the content of deoxynivalenol (DON) mycotoxin has been found to be correlated with the level of resistance to FHB. Despite many studies the effective markers for the selection of barley genotypes with increased resistance to FHB have been not developed so far. Different types of molecular markers were employed to barley genotyping and map construction, starting from the low-density map (e.g. RFLP markers) to high-density map based on single nucleotide polymorphism (SNP) markers. SNPs have been widely used in molecular studies because of their ubiquitous presence in high numbers, uniform distribution and biallelic nature. Moreover, single nucleotide mutation within the gene may be responsible for changes in gene function and may lead to the phenotypic differences, as a consequence. Thus, SNP discovery is a prerequisite for effective identification of linkage between genetic background and important agronomical traits. This study is focused on the pleiotropic effects of the earliness genes in respect to the barley susceptibility to pathogen disease caused by fungi of the *Fusarium* genus. Studied plant material consisted of 60 cultivars and 200 recombinant inbred lines (RIL) of spring barley – being half-siblings. The genetic background of RILs was assessed by high-throughput SNP genotyping platform (iSelect platform with 7842 markers). Plant material was examined in field conditions in the completely randomized block design with three replications. Barley genotypes were inoculated by spores of *Fusarium avenaceum, F. graminearum and F. culmorum* two days before heading. In addition to the main phenotypic traits (e.g. plant height, spike characteristic, grain yield) the level of Fusarium head blight infection was assessed by scoring infected kernels and estimation of the deoxynivalenol (DON) mycotoxin content with the used of Ridascreen DON kit. Functional annotation of SNP markers with respect to the published sequence of the barley genome will allow identifying genes determining the FHB resistance.

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The influence of the genotype, developmental phase of the spike and developmental stage of microspores on the induction of androgenesis in anther cultures of rye (*Secale cereale* L.)

Sylwia Mikołajczyk[a], Zbigniew Broda[a], Dorota Weigt[a], Agnieszka Tomkowiak[a] and Jan Bocianowski[b]

[a] *Department of Genetics and Plant Breeding, Poznan University of Life Sciences, Poland*
[b] *Department of Mathematical and Statistical Methods, Poznan University of Life Sciences, Poland*

sylviam@au.poznan.pl

Rye is a species where haploid induction and regeneration of green plants in anther cultures causes many problems. The aim of the study was to search for rye genotypes with high effectiveness of induction of androgenesis and regeneration of green plants and to determine the optimal developmental stage of the spike and microspores to start anther cultures.
Donor plants of winter rye (13 population cultivars and 30 genotypes which were breeding materials of different origins) grew in a greenhouse. Rye spikes were cut between stage 49 and stage 52 in the BBCH scale. Then the following two biometric traits were measured: the distance between the sub-flag leaf and the flag leaf and the lengths of anthers in the central part of the spike. The anthers collected for measurement were used to make smear specimens stained with acetic carmine and the developmental stage of rye microspores was determined. After biometric measurements and preparation of smear specimens the spikes were subjected to thermal shock – they were stored at a temperature of 4^{o}C for three weeks. The anther cultures of 43 rye genotypes under study were grown on a C17 medium containing two combinations of growth regulators: C17+2mg/l 2,4-D and C17+1mg/l 2,4-D+1mg/l of dicamba. Univariate and multivariate analysis of variance (ANOVA) in a random arrangement was used to assess the statistical significance of differences between the genotypes, the developmental stage of the spike and the medium as well as interactions between them.
The assessment of the effectiveness of induction of androgenesis and regeneration of plants in the anther cultures of 43 rye genotypes led to the following conclusion. The cultivars Amilo, Antonińskie, Arant, Dańkowskie Amber, Pastar, Rostockie and breeding materials PHR9/15, PHR65/15, PHR75/15, S1128/14, S1142/14, S2550/14 were characterised by a statistically significant higher response in the rye anther cultures and regeneration of green plants. There were no statistically significant differences between variants of the C17 medium (C17 + 2mg/l 2,4-D and C17 + 1mg/l 2,4-D + 1mg/l of dicamba) or a statistically significant interaction between the genotype and medium for two parameters of androgenesis (the number of reacting anthers and the number of green plants regenerated). The induction of androgenesis and regeneration of plants in the rye anther cultures were statistically significantly influenced by the plant material and the interaction between the plant material and the stage of spike development. The rye cultivars were characterised by the greatest capability of androgenesis and regeneration of plants from microspores at early-mid-mononuclear and mid-mononuclear stages and from anthers at an average length of 5.1mm. The breeding materials exhibited induction of androgenesis and regeneration of plants from more developed microspores – ranging from the mid-mononuclear stage to the first mitotic division of microspores, and from longer anthers – ranging from 5.8 to 6.4mm.

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Novel dwarfing gene in triticale – influence on the straw length and key gibberellin biosynthesis pathway genes expression

Michał Nowak[a], Justyna Leśniowska-Nowak[a], Karolina Dudziak[a], Daniela Gruszecka[a], Aleksandra Gogół[a] and Krzysztof Kowalczyk[a]

[a] *Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin, Poland*

michal.nowak@up.lublin.pl

In recent years, global production and the significance of triticale have shown an upward trend. Lodging is one of the main concern negatively affecting triticale grain yield and quality. The best way to overcome this problem is to breed new cultivars with dwarfing genes introduced, which would effectively reduce the straw length. The aim of the present study was to characterize a novel dominant dwarfing gene identified in spontaneous triticale mutant. The object of analysis was the spontaneous dwarf mutant identified in the population of hybrids derived from triticale crossing with wild goatgrass species *Aegilops juvenalis* (Thell.) Eig. Allelism tests were performed to determine the allelic relationship of the dwarfing gene studied with *Ddw1* and exclude the occurrence of the same dwarfing gene in the analysed mutant genetic background. Dwarf mutant plants were crossed with semi-dwarf Polish triticale cultivars containing the *Ddw1* dwarfing gene. Obtained results revealed that the examined gene is non-allelic to the dominant *Ddw1* gene, however, the additive effect of gene activity was noticed. The aim of presented study was determination of the novel dwarfing gene presence on triticale plants straw length. Moreover, its influence on expression of the genes encoding three major enzymes involved in bioactive gibberellins biosynthesis (GA20ox, GA3ox and GA2ox) was analysed. Obtained results revealed, that the straw length of dwarf mutant plants was shortened by 22% in comparison to typical plants derived from the same cross. Subsequent analysis of the length of individual internodes showed that the total number of internodes in dwarf plants was reduced to four and significant shortening was noticed for peduncle and third internode. Moreover, presence of analysed novel dwarfing gene did not cause significant alteration of diameter for none of the internodes. Analysis of gibberellin biosynthesis pathway genes expression by means of qPCR technique revealed, that presence of analysed dwarfing gene cause modification of their transcription pattern. For *GA20ox* and *GA3ox* genes, encoding enzymes responsible for transformation of precursors into bioactive gibberellin forms, the decrease of transcript level in dwarf mutant was observed in comparison to tall form. For *GA2ox* gene, encoding enzyme responsible for degradation of bioactive gibberellins, upregulation of transcription was noticed. These results indicate, that one of the factors determining dwarf phenotype of mutant triticale plants is deficiency of bioactive gibberellins, caused by alteration of key gibberellin biosynthesis pathway genes expression level.

Avena sterilis L. genotypes as a potential source of resistance to Oat crown rust

Sylwia Okoń, **Tomasz Ociepa**, Edyta Paczos-Grzęda, Sylwia Sowa, Agnieszka Ostrowska, Aneta Koroluk and Krzysztof Kowalczyk

Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin, Poland

tomasz.ociepa@up.lublin.pl

Crown rust, caused by *Puccinia coronata* Cda. f.sp. *avenae* is the most widespread and harmful fungal disease of oat. Crown rust not only reduces the yield (up to 50%) and grain quality but also increases lodging. The introduction of effective resistance genes into cultivars by natural selection is the most effective and environmentally friendly method of controlling this disease. Wild species with similar genomic structure as common oat may be a valuable source of specific racial resistance to *P. coronata*. The aim of this study was the identification of potential resistance sources to oat crown rust among *Avena sterilis*.

In the experiment 15 genotypes of *Avena sterilis* provided by Genebank Gatersleben (Germany) were used. Evaluation of resistance was carried out using host-pathogen tests based on 25 pathogen isolates collected from experimental plots located in different parts of Poland. Host-pathogen tests were carried out on the first leaves of 10-day-old seedlings of tested wild oat genotypes. Leaves fragments of analyzed genotypes were placed on 12-well culture plates filled with agar (0.6%) containing benzimidazole (3.4 mM) and were inoculated in an inoculation tower with about 500-700 crown rust urediniospores per 1 cm^2. Then the dishes were incubated in a phytotron at about 18°C and illuminance of approximately 4 kLx. The effect on the leaves was determined ten days after inoculation using the numeric 0-4 scale of Murphy (0 – no visible reaction, 1 – chlorotic or necrotic flecking, 2 – small pustule surrounded by chlorosis, 3 – moderately large pustules surrounded by extensive chlorosis, 4 – large to moderately large pustules with little or no chlorosis). The cultivar „Kasztan", which is fully susceptible to crown rust infection, was used as an internal standard. To gather full information about the response of selected genotypes to stress caused by the pathogen the genotypes were tested on experimental plots under natural infection.

The results showed differences in response of selected *Avena sterilis* genotypes to crown rust isolates. The highest level of resistance in the seedling stage showed AVE 1935 and AVE 2532 genotypes, which were resistant to 96% of used isolates. However, observations in the seedling stage did not always correlate with adult plants' resistance. Under natural infection conditions the AVE 2532 genotype was resistant in the adult plant phase, while AVE 1935 genotype demonstrated intermediate resistance to the pathogen infection. Noteworthy is the high resistance in the adult plant stage showed by genotypes that in seedlings stage were infested by almost half (AVE 245, AVE 446), or the majority of tested isolates (AVE 1983). The adult plant resistance is defined as the resistance, which is not revealed in the seedling stage. The resistance mechanism has not yet been fully understood. It is believed to be the hypersensitive response of plant to pathogen. From the breeders point of view high resistance in the adult plant stage is more relevant than that in the seedling stage. Therefore, the analysis of both the seedling stage and the adult plant stage is an essential aspect of studies on the sources of resistance to fungal diseases.

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Analysis of molecular mechanism of resistance to *Fusarium* head blight in triticale at the proteome level

Dawid Perlikowski[a], Halina Wiśniewska[a], Joanna Kaczmarek[a], Tomasz Góral[b], Piotr Ochodzki[b], Michał Kwiatek[a], Maciej Majka[a], **Adam Augustyniak[a]** and Arkadiusz Kosmala[a]

[a] *Institute of Plant Genetics of the Polish Academy of Sciences, Poland*
[b] *Plant Breeding and Acclimatization Institute - National Research Institute, Poland*

aaug@igr.poznan.pl

Triticale was used here as a model to recognize new components of molecular mechanisms of resistance to *Fusarium* head blight (FHB) in cereals. *Fusarium*-damaged kernels of two lines distinct in levels of resistance to FHB were applied into proteome profiling using two-dimensional gel electrophoresis to create protein maps and mass spectrometry to identify the proteins differentially accumulated between the analyzed lines. The comparative analyses indicated a total of 23 spots that showed differences in protein abundance between the more resistant and more susceptible triticale lines after infection with *F. culmorum*. The majority of the proteins were involved in a cell carbohydrate metabolism, stressing the importance of this protein group in a plant response to *Fusarium* infection. The increased accumulation levels of different isoforms of plant beta-amylase were observed for a more susceptible triticale line after inoculation. The more resistant line was characterized by a higher abundance of alpha-amylase inhibitor CM2 subunit. The inhibition of pathogen alpha-amylase activity could be one of the most crucial mechanisms to prevent infection progress in triticale, as it was observed earlier in wheat (Perlikowski et al. 2014).

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Yield component analysis of 13 spring barley genotypes in a two row „honeycomb" system

Ioana Porumb[ab] and Russu Florin[a]

[a]*Agricultural Research and Development Station Turda, Romanina*
[b]*University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania*

ioanaporumb18@yahoo.com

Barley is one of the most ancient cultivated plants which played an important role in the development of human civilization, agricultural sciences, physiology, genetics and plant breeding (Ullrich, 2011). Currently, barley is the fourth important cereal in the world after wheat, corn and rice, regarding yield and cultivated area.
Fasoula and Fasoula (1997, 2000) suggest the importance of stress conditions such as minimum plant density, which can avoid competition between plants to optimize the effective selection and growing the productive potential of the plant.
This paper aims to clarify the biological potential of local and foreign cultivars of spring barley, and it also studies the traits of yield components. To achieve the aim of this paper we used a noncompetitive system between plants, known as honeycomb designs or comb system. Honeycomb was first proposed in 1973 by Fasoulas as a selection method for heterogeneous populations of self-pollinating plants, but it can be used to obtain good results in cross pollinated plants selection. This method aims to eliminate the competition factor between plants and minimize the negative effects of hidden correlation between production and competitive capacity (Fasoulas and Fasoula 2000). Two grains of barley were planted at each position and after postemergence one plant was eliminated. Distance between plants in the row was 50 cm, and 43.3 cm between rows. Researches were performed in the years 2013-2014 and the genotypes used were: Daciana, Turdeana, Romanița, Capriana, Jubileu (S.C.D.A Turda), Adina, Farmec (S.C.D.A. Suceava), Thuringia (Saaten Union), Marlen, Derkado, Tremois (Limagrain), Mauricia and Sewa (KWS). The main components of production that have been studied are: tillering ability, number of grains of the main ear, grain weight of main spike, grain weight / plant and harvest index.
The highest capacity of tillering was observed for the cultivar Turdeana where an average number of 22 tillers were observed in the two experimental years. The greatest grain weight/spike was observed for cultivars Daciana, Turdeana and Romanița, which have a high biological potential.

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Role of *HvCKX5* and *HvCKX4b* genes in growth and reproductive development of barley

Izabela K. Rajchel[a], Karolina Słomińska[a], Wacław Orczyk[b] and Anna Nadolska - Orczyk[a]

[a] *Plant Breeding and Acclimatization Institute - National Research Institute, Department of Functional Genomics*
[b]*Department of Genetic Engineering, Radzikow, Blonie, Poland*

i.rajchel@ihar.edu.pl

Cytokinins are a class of phytohormones that promote and influence numerous developmental processes in plants. Cytokinin oxidases/dehydrogenases (*CKX*) catalyze the irreversible degradation of the cytokinins in a single enzymatic step by oxidative side chain cleavage. The enzymes are encoded by a multigene family of *CKX* genes. Their expression is tissue specific and developmentally regulated. To date the sequences of 11 fully or partly annotated *HvCKX* genes are known, but their role in growth and reproductive development have been reported for two of them.

In current research, we focused on two genes: *HvCKX4b* and *HvCKX5*. We applied expression profiling and stable, interference-based gene silencing technology (RNAi) to characterize them. A quantitative analysis of expression of the *HvCKX* genes were performed in different tissues/organs by the RT-qPCR technique. The silencing cassettes were cloned in the two-step protocol of Gateway technology, in which fragments of target genes were inserted into entry vectors (pCR™8/GW/TOPO^R) and then transferred to the destination vectors (pBract207) in sense and antisense orientation. The correct orientation of the insertions was confirmed by restriction enzyme analysis and DNA sequencing. The obtained vectors were electroporated to competent *Agrobacterium tumefaciens*, AGL-1 strain. The *Agrobacterium* - mediated transformation of barley immature embryos of spring cultivar Golden Promise and breeding line STH7308 was conducted. 22 transgenic plants of Golden Promise were selected from 16 independent callus lines and 19 transgenic plants of STH7308 from 14 different transformation events. For silencing the *HvCKX4b* gene we performed four cycles of transformation and selected 17 transgenic plants of Golden Promise (from 13 independent callus lines) and 19 positive plants (from 14 different transformation events). The transgen integration was confirmed by PCR with specific primers designed for T-DNA of the vector. The expression profiles of both analysed *HvCKX* genes were performed in different tissues/organs of developing wild-type plants. The highest level of the *HvCKX5* expression in Golden Promise was detected in leaf tissue while lower expression - in roots and spikes 14 days after pollination (DAP). In STH7308 the highest level of the *HvCKX5* expression was observed in spikes 14 DAP and lower in leaf tissue. In case of *HvCKX4b* gene, the levels of expression were the highest in roots and 14 DAP spikes in both, Golden Promise and STH7308.

Based on these results, a three-week leaf tissue was chosen for the quantitative analysis of silencing of *HvCKX5* gene expression in the T_0 generation of Golden Promise and STH7308. Reduced expression of the *HvCKX5* gene was detected in 82% of transgenic Golden Promise (18 plants) and in 74% (14 plants) of transgenic STH7308. The relative silencing ranged from 100-16% for Golden Promise and 99% -7% for STH7308.The measurement of the *HvCKX4b* gene expression in spikes 14 DAP in T_0 transgenic Golden Promise and STH7308 lines was not possible. We observed a significant decrease in kernel germination/setting the seeds. The *HvCKX4b* gene expression in roots and spikes 14 DAP as well as the *HvCKX5* expression in leaves, roots and 14 DAP spikes as well as analysis of phenotypic traits in T_1 generation of transgenic lines are in the progress and would be presented.

Genetic structure and identification of genetic regions associated with root traits in rice under drought stress using AFLP markers and hydroponic culture

Hossein Sabouri[a], Abbas Biabani[a], Mahnaz Katouzi[b], Ahmad Reza Dadras[c], Sharifeh Mohammad Alegh[a], Mohammad Javad Behrozbeh[a], Galdi Mohammad Bahlakeh[a], Reza Karim Koshteh[a] and Atefeh Sabouri[c]

[a] *Department of Plant Production, University of Gonbad Kavous, Iran*
[b] *Department of Plant Breeding, Gorgan University of Agriculture Science and Natural Resource, Iran*
[c] *Department of Plant Breeding, University of Guilan, Iran*

hossein.sabouri@gonbad.ac.ir

Developing a deep root system is an important strategy for avoiding drought stress in rain-fed rice. However, the roots are usually not accessible in conventional breeding programs and marker assisted selection would be a way to select these difficult-to-phenotype traits. The aim of this study was to detect, genomic regions related to the tolerance of roots to osmotic stress. A study was conducted using 192 rice genotypes (Landrace, introduced and improved) under drought stress. Genotypes planted in hydroponic condition. To apply osmotic stress was used Mannitol with -5 bar concentration in the seedling stage. Shoot, root and plant mass, root thickness, length of shoot and root were recorded in the completely randomized design with three replications on 7th, 14th, 21th, 28th and 35th days after transferring to hydroponic culture. The averages of replications for the traits were used as phenotype data in association analysis. Genotyping of the population was performed using primer combinations of *Eco*RI and *Mse*I restriction enzymes. To identify genomic regions associated with loci controlling the different traits, five statistical models with two GLM and MLM procedures were used with TASSEL software. The MLM model was used to eliminate false positives caused by population structure and kinship among individuals. The results revealed the marker of E-*AGT*-M-*AAC*-3 linked to shoot length 21th, 25th days explained 43.07 and 45.11% of phenotypic variation. Also, this marker related to root length in 7th and 21th days and explained 27.11 and 31.79% of phenotypic variation. The markers of E-*ATC*-M-*AAC*-9, E-*AGT*-M-*AAC*-8 and E-*ATC*-M-*AAC*-9 for shoot weight, root weight and biomass explained 77.37%, 16.33% and 12.91% of phenotypic variation, respectively. Considering that above markers explained significant percentage of the phenotype variations can be used as candidate markers in further studies. These results would be used for conversion to SCAR markers after validation QTL and linked marker and be used in marker assisted selection for drought tolerance.

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A comprehensive research in genetic structure of drought tolerance in Iranian RIL population

Hossein Sabouri[a], Abbas Biabani[a], Mahnaz Katouzi[b], Ahmad Reza Dadras[c], Sharifeh Mohammad Alegh[a], Mohammad Javad Behrozbeh[a], Galdi Mohammad Bahlakeh[a], Reza Karim Koshteh[a] and Atefeh Sabouri[c]

[a] *Department of Plant Breeding, Gorgan University of Agriculture Science and Natural Resource, Iran*
[b] *Department of Plant Production, University of Gonbad Kavous, Iran*
[c] *Department of Plant Breeding, University of Guilan, Iran*

hossein.sabouri@gonbad.ac.ir

Drought serves as a major abiotic stress of rice, and in the tolerance systems to drought stress good root and shoot growth has been linked strongly with drought avoidance. We mapped QTLs controlling root and shoot-specific morphological traits in rice (*Oryza sativa* L.) under normal and stress conditions using 96 lines obtained from crossing two cultivars Sepidroud and Anbarboo at the research field located at the Gonbad Kavous University in 2011. A linkage map was developed using 123 microsatellites and 261 AFLP markers. The map covered 1950.4 cm of the rice genome. Under normal condition, the identified QTLs at intervals of E-*AGT*-M-*AAC*-7_RM3520 on chromosome 1, E-*AAG*-M-*AGT*-3_RM1359 on chromosome 4 and RM276_E-*ATT*-M-*AGT*-3 on chromosome 6 controlled multiple traits. QTLs controlling root dry weight, root fresh weight and root number on chromosome 7 were found as co-located. Some Major QTLs were detected, including QTLs controlling root volume (qRVN-2a, qRVN-4a and qRVN-4b) and root number (qRNN-4). These major QTLs explained up to 20% of the phenotypic variation. To simulate drought, plants were irrigated in interval 25 days from maximum tillering stage to maturity. Under drought stress condition, the E-*AAT*-M-*AAC*-1_E-*AAT*-M-*AGA*-13 interval on the chromosome 2, E-*AAG*-M-*AGT*-3_RM1359 interval on the chromosome 4 as well as E-*ATT*-M-*AAC*-9_E090-M140-14 on the chromosome 9 were detected as controlling genome regions in multiple traits. Colocation of traits indicates a similar genetic control under stress condition. Across different traits 15 major QTLs including qSWD-2a, qSWD-4, qEWD-2, qEWD-4, qERD-2a, qERD-4, qEVD-2, qEVD-4a, qRWBD-2, qRWBD-4, qRWAD-2a, qRWAD-4a, qRND-2a, qRND-4a and qPPN-9 were detected, explained above 20% phenotypic variations in traits. The mentioned regions are potentially candidate for marker-assisted selection for draught tolerance after validation in other environments and populations. Therefore, identified major QTLs in this report after verification can be used in marker-assisted selection breeding plans.

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The image-based phenotyping to analyze the genes involved in salt stress tolerance of rice

Hye-Jin Yoon, Kyung Hwan Kim, Hyeon So Ji, In-Chan Choi and Taek-Ryun Kwon

Gene Engineering Division, Department of Agricultural Biotechnology, National Institute of Agricultural Sciences, RDA, Korea

hyejinyoon@korea.kr

Precise quantification of traits contributes to the genetic improvement of crop plants. In recent years, a large amount of genomic data obtained by high-throughput genotyping at low cost has played a key role to expand the area of plant phenomics. The advance of sensors, system technologies also lead the way of new plant phenotyping applications. It supports the development of the technologies such as non-invasive imaging, information technology based computing, high-performance automation in phenotyping. However, phenotyping is still a manually performed activity, and different from each species, environment, and trait. It is often destructive, labor-consuming, and can be sensitive to environmental changes, and not objective.

There is a strong demand for phenotypic data of high quality. Digital phenotyping enables the development of high throughput non-invasive imaging technologies including color imaging for biomass, plant structure, leaf health with good accuracy.

Here we described the image-based technology as applied to alleviate the bottleneck for the development of high-throughput phenotyping platforms. Several trials to measure salt stress responses of rice plantlets based on image data by using 3D scanalyzer are underway to develop the physiological parameter for the next-level of phenotyping.

Metabolite profiling of diverse rice germplasm and identification of conserved metabolic markers of rice roots in response to long term mild salinity stress

In Sun Yoon[a], Taek Yun Kwon[a], Hay Ju Han[a] , Eunjung Bang[c] , Beom Gi Kim[a] and Myung Hee Nam[b]

a Gene Engineering Division, National Institute of Agricultural Sciences, Republic of Korea
b Environmental Risk and Welfare Research Team, Korea Basic Science Institute, Republic of Korea
c Omics System Research Team, Korea Basic Science Institute, Republic of Korea

isyoon@korea.kr

The sensitivity of rice to salt stress is greatly depends on growth stages, organ types and cultivars. Especially, the roots of young rice seedlings are highly salt sensitive organ that limits plant growth even under mild soil salinity condition. In an attempt to identify metabolic markers of rice roots responding to salt stress, metabolite profiling was performed by ^{1}H-NMR spectroscopy in 38 rice genotypes that varied in biomass accumulation under long-term mild salinity condition. Multivariate statistical analysis showed separation of the control and salt-treated rice roots, and rice genotypes with differential growth potential. By quantitative analyses of ^{1}H-NMR data, five conserved salt-responsive metabolic markers of rice roots were identified. Sucrose, allantoin and glutamate accumulated by salt stress, whereas the levels of glutamine and alanine decreased. A positive correlation of metabolite changes with growth potential and salt tolerance of rice genotypes was observed for allantoin and glutamine. Adjustment of nitrogen metabolism in rice roots is likely to be closely related to maintain growth potential and increase stress tolerance of rice. Supported by grants from the Next-Generation BioGreen 21 Program (SSAC, PJ00951406) of the Rural Development Administration, Republic of Korea.

Next generation evolutionary breeding for sustainable agriculture: experiences with barley

Lorenzo Raggi[a], Salvatore Ceccarelli[b] and Valeria Negri[a]

[a] *Department of Agricultural, Food and Environmental Sciences, University of Perugia, Italy*
[b] *Rete Semi Rurali, Firenze, Italy*

lorenzo.raggi@gmail.com

Traditional plant breeding programmes produced many successful genetically uniform cultivars that are high yielding in high input production systems. However, an increased request for safe food as well as of environmentally friendly agriculture strongly suggests the need to shift, at least in part, from the conventional way of producing food to organic (OA) and low-input (LI) agriculture. The application of Next Generation Genotyping (NGG) technology to heterogeneous populations developed through Evolutionary Breeding (EB) can dramatically reduce time and costs for the development of new varieties for OA and LI by allowing a rapid and precise identification of genotypes adapted to different environments.
We developed an evolutionary population (a composite cross named AUT DBA) through an EB program for LI agriculture. A new population (named *mix48*) was built by mixing the seed of 48 lines extracted from AUT DBA and characterized by high grain yield and a favourable combination of traits relevant for OA and LI. The *mix48* population was then multiplied for three successive years at five locations characterized by different climatic conditions obtaining five evolutionary populations.
The aim of our work was: i) to evaluate the output of the EB program from which genetically diverse populations and pure lines were obtained, and ii) to study the genetic and morpho-phenological diversity evolution of *mix48* population.
For each of the traits we studied, pairwise differences between *mix48* and each evolutionary population were tested. The same materials, plus a sample of the initial parental populations (PPs) intercrossed to obtain the AUT DBA, were genotyped (more than 400 SNPs). Genotyping data were used for a parentage analysis of 440 individuals sampled from the five evolutionary populations. The average contribution of each one of the different PPs to the genetic constitution of *mix48*, and of each evolutionary populations was estimated by using a Bayesian clustering method.
Individuals most frequent in the AUT DBA showed a different plant architecture compared with control varieties and most of the EB products were high yielding in LI and OA. Differences among the evolutionary populations were detected for all the traits. The genomic analysis (250K data points) showed differences in the genetic constitution of the evolutionary populations and that the PPs differently contributed to their genetic constitution. Finally, different genotypes significantly increased their frequency in different environments.
Our findings suggest that alternative breeding programs like EB when supported by NGG data can be very useful in identifying lines and heterogeneous materials adapted to different environments. Genomic analysis can also help in the identification of key genomic regions involved in the control of adaptation, which is particularly relevant under the current climate change scenario.

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Parallel flash and poster presentations: Fodder crops and Maize

Separation of endophytic and epiphytic phyllosphere bacterial communities of *Lolium spp*.

Martina A Birrer, Franco Widmer and Roland Kölliker

Agroscope Institute for Sustainability Sciences, Zurich, Switzerland

martina.birrer@agroscope.admin.ch

Ryegrasses (*Lolium* spp.) are important components of pastures and meadows and have great impact on grassland-based agriculture. They are vulnerable to various bacterial and fungal pathogens including *Xanthomonas translucens* pv. *graminis* or *Puccinia coronata*. Great efforts have been made to study the pathogen-*Lolium* interaction and to identify host-resistance and pathogen-virulence. However, apart from fungal endophytes, little attention has been paid to interactions of *Lolium* spp. with symbiotic or commensal microbes. The plant microbiome, i.e. the entity of plant associated microorganisms, has been shown to substantially influence plant performance and resistance against pathogens. While considerable effort has been made to describe the root microbiome of various plants, little information is available on the leaf microbiome of grassland species.

The overall aim of this project is to gain a better understanding of the diversity of bacterial phyllosphere communities associated with *Lolium* spp. in grassland. In a first step, we aimed at separating epiphytic from endophytic communities and adapting a sequencing approach based on the 16S ribosomal RNA gene.

Plant leaves were washed in a phosphate buffer combined with a sonication treatment. The adhesion of bacteria to plant surfaces was analysed. Repeated washing distinctly reduced the amount of bacteria detected in the buffer solution. However, ribosomal intergenic spacer analysis (RISA) revealed no apparent changes in bacterial community composition.

In order to enable massive parallel sequencing of bacterial communities from whole plant tissue, universal 16S rRNA gene primers were applied. The PCR was supplemented with specifically designed peptide nucleid acid (PNA) probes to reduce the amplification of plant organelles. PNAs are designed to bind to mitochondria (mPNA) or chloroplast (pPNA) DNA and function as sequence specific PCR blockers. The amplicons were sequenced on the Illumina MiSeq platform.

First results indicate a successful reduction of amplicons from plant organelles by adding PNAs to the PCR. An eightfold increase of bacterial reads could be achieved in samples where PNAs were added. In total, 247 bacterial operational taxonomic units (OTU) were derived from 12’280 bacterial reads, including known phyllosphere genera such as *Pseudomonas, Sphingomonas, Methylobacterium* and *Agrobacterium*.

Furthermore, no loss of OTUs could be observed due to PNA addition. Thus, PNAs offer a powerful tool to decrease organelle reads in plant samples and therefore allow to detect microbial communities in and on leaf material. This opens the way for detailed investigations of the *Lolium* leaf microbiome in different environments and on different genotypes.

Freezing tolerance of diploid versus tetraploid in perennial ryegrass

Andrius Aleliūnas[a], Kristin H. Gylstrøm[b], Petter Marum[b] and **Gintaras Brazauskas**[a]

[a]*Institute of Agriculture,Lithuanian Research Centre for Agriculture and Forestry, Lithuania*
[b]*Graminor AS, Norway*

gintaras.brazauskas@lzi.lt

Freezing tolerance (FT) is one of the major factors determining overwintering potential in plants, thus limiting yield in freeze-susceptible perennial crops. Perennial ryegrass (*Lolium perenne* L.) is a preferred fodder species in Europe, however, poor overwintering restricts it is wider use at Northern latitudes. A test set of 154 diverse accessions of perennial ryegrass obtained from various Gene Banks was subjected to an artificial freezing test to screen for FT. All accessions were revised for ploidy level by flow cytometry, revealing 129 diploid and 25 tetraploid accessions in the test set. Most (92%) of the genotypes were of European origin, however, genotypes from US, Canada, Turkey, Japan and Kyrgyzstan were also included. Sixty seeds per accession were sown into a perlite/vermiculite (50:50, v/v) substrate and grown hydroponically for 1 month and then were cold-hardened for 2 weeks at 2 °C, while maintaining a 12/12 h photoperiod at 200 μmol m^{-2} s^{-1} light intensity (PAR). Cold-hardened plants were subjected to freezing by slowly (2 °C h^{-1}) lowering temperature to the target temperature (measured in the substrate at crown depth) that was maintained for 24 hours at. Freezing test was repeated for a total of six target temperatures of -4, -6, -8, -10, -12 and -14 °C. Plant survival was scored after 3 weeks of plant recovery in the greenhouse and LT_{50} values were subsequently estimated for each accession applying a GLM model.

Freezing test revealed substantial variation for the freezing tolerance in the test set of perennial ryegrass with LT_{50} value ranging from -9.81 to -5.37 °C (median LT_{50} value of -7.95 °C). There was a significant ($p < 0.001$) difference in the freezing tolerance between diploid and tetraploid accessions with an average LT_{50} value for the diploid accessions reaching -8.18 ± 0.80 °C, while the tetraploid accessions had an average LT_{50} value of only -6.09 ± 0.46 °C. Most freezing tolerant accessions were ecotypes of Central European origin, specifically from Poland, Romania, Ukraine and Hungary. These most freezing tolerant populations were also identified to be diploid, while the tetraploid populations in our panel were classified as freeze-susceptible ones.

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Sequencing and comparative analysis of mitochondrial genomes of fertile and male-sterile lines in perennial ryegrass (*Lolium perenne* L.)

Istvan Nagy, Stephen Byrne[a], Ian Max Møller and Torben Asp

Department of Molecular Biology and Genetics, Aarhus University DK-4200 Slagelse, Denmark
[a] Crops Research Centre, Teagasc Crops Environment and Land Use Programme, Oak Park, Carlow, Ireland

Istvan.Nagy@mbg.au.dk

Plant mitochondrial genomes are large and variable in size with substantial amounts of non-coding regions. Sequence parts of plant mitochondrial genomes represent "promiscuous" DNA of nuclear and plastid origin, as well as sequence regions that are likely be obtained through horizontal transfer from mitochondria of other species. While angiosperm mitochondrial genomes exhibit extremely slow rates of nucleotide substitutions, they are subjected to a rapid structural evolution. The complete mitochondrial sequence and a circular molecular map for the fertile ryegrass line F1-30 has been recently published by our group[1]. In addition, we accomplished complete mitochondrial genome sequences of two cytoplasmic male-sterile (CMS) lines and in a fertile maintainer line in *Lolium perenne* by *de novo* hybrid Next Generation Sequencing approaches using Roche 454 Pyrosequencing and Pacific Biosciences Single Molecule Reads technologies. For comparative transcriptome analyses, RNAseq studies were carried out using immature inflorescence tissues from fertile and male sterile lines. Genome sequencing reveals in parallel existing mitochondrial sub-genomes with extensive structural rearrangements in all investigated ryegrass genotypes. On the other hand, mitochondria of all investigated perennial ryegrass lines share the same basic sequence content between highly conserved sequence blocks, indicating that sub-genomic molecules are likely to arise from a circular master molecule through intra- and/or inter-molecular recombination. Sequence alignments and short-read mapping provide evidences that mitochondrial sub-genomes are complementing and stoichiometrically balanced in each line. These findings are congruent with results from earlier electrophoretic and electron-microscopic studies proposing the coexistence of a recombination-dependent mode of replication with a presumably recombination-independent rolling-circle mode of replication in higher plant mitochondria[2]. *Ab initio* gene predictions and RNAseq data showed that all canonical protein coding genes are present in the mitochondrial genomes of all investigated ryegrass lines (two fertile and two CMS lines). The majority of protein coding sequences are highly conserved across the four lines. However, in case of a few genes post-transcriptional modifications (RNA editing) might result in different Open Reading Frames in fertile and male-sterile lines. Sequence-level comparisons to other published grass mitochondrial genomes suggest that the mechanisms of male sterility in the two investigated perennial ryegrass CMS lines and the mechanisms of K-type CMS found in wheat[3] might share analogies.

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Root system performance under water deficit conditions in *Lolium multiflorum/Festuca arundinacea* introgression forms

Dawid Perlikowski[a], Adam Augustyniak[a], and Arkadiusz Kosmala[a]

[a]*Institute of Plant Genetics of the Polish Academy of Sciences, Poland*

dper@igr.poznan.pl

Water deficit is one of the major stress factors that influence growth and development of plants during their life cycle. The tolerance to water deficit in plants, including forage grasses, is closely associated with changes in morphology and metabolic processes of roots. A highly developed root system and its efficient functioning is a key element required in plants to survive and maintain homeostasis during the severe water deficit. In addition, an efficient stress signalling system, allowing for a rapid plant reaction and its adaptation to the new conditions, is also essential. All these aspects emphasize the high importance of roots as organs that are the first line of defence for plants during water deficit stress. *Lolium multiflorum* (Italian ryegrass) is a forage grass species characterized by a high forage quality, but a relatively low tolerance to biotic and abiotic environmental stresses. On the other hand, *Festuca arundinacea* (tall fescue) is a species with a high level of abiotic stress tolerance, particularly drought tolerance, but it does not match *L. multiflorum* in terms of productivity and quality. *F. arundinacea* is regarded as a model plant in the *Lolium-Festuca* grasses for the research on drought tolerance mechanisms, mainly due to a highly developed root system. *L. multiflorum* and *F. arundinacea* hybridization enables the assembly of complementary characters of both species within a single genotype. In our previous studies, we selected two *L. multiflorum/F.arundinacea* introgression forms, characterized by a distinct level of drought tolerance under simulated field conditions (long-term drought, 14 weeks), a different photosynthetic capacity under simulated drought in the pots (short-term drought, 11 days) and a different ability to recover cellular membranes after re-hydration. The introgression form 7/6 with a lower yield during long-term drought and re-growth potential after re-hydration was characterized by more efficient photosynthesis during short-term drought. In turn, the introgression form 4/10 with a higher yield under drought and recovery potential in the field was simultaneously shown to have better mechanism of cellular membrane regeneration after stress cessation in the pots (Perlikowski et. al. 2014). Herein, we hypothesize that the root system could be a crucial component responsible for a higher drought tolerance observed in the form 4/10 in the field. No crucial differences between the analyzed introgression forms with respect to the important drought tolerance indicators relative water content and electrolyte leakage were observed during short-term drought in pots with a limited soil space for a proper development of roots. To confirm this hypothesis and to evaluate a root system functioning under drought, we performed the experiments in the simulated water deficit conditions in pipe systems during six weeks. The obtained results revealed that the introgression form 4/10 was in fact characterized by a faster response to drought conditions, which was manifested by more efficient root growth after initiation of drought conditions, a higher root to shoot ratio, a slower retardation of shoot growth and a higher yield potential compared to the introgression form 7/6.

Perlikowski D., Kosmala A., Rapacz M., Kościelniak J., Pawłowicz I., Zwierzykowski Z. (2014). Influence of short-term drought conditions and subsequent re-watering on the physiology and proteome of *Lolium multiflorum*/*Festuca arundinacea* introgression forms with contrasting levels of tolerance to long-term drought. Plant Biology 16: 385-394.

Improvement of drought tolerance of forage perennial ryegrass by breeding of root characteristics and deep root production

Dejan Sokolović, Snežana Babić, Jasmina Radović, Vladimir Zornić and Mirjana Petrović

Institute for forage crops, Kruševac, Serbia

dejan.sokolovic@ikbks.com

Global weather changing and water deficit have negative impact on agriculture. The majority of most productive and quality cool season grasses are highly drought susceptible. One way to bypass this problem in forage production is cropping of drought resistant or tolerant cultivars. Traditionally, forage plant breeding has been focussed on improving the economically most important traits, i.e. yield, dry matter quality, diseases resistance and seasonality of production, but recent years, drought tolerance as breeding criterion has become extremely important. Since now, the majority of attempts to develop more drought-tolerant grass plants have concentrated on determining plants with prolonged field persistency, or evaluating genotypes in dry conditions, both in the field or rain shelters, which is time consuming and expensive. In grasses, drought tolerance is closely related to the distribution and penetration of root systems into the deeper portions of the soil, since they need to keep some root contact with ground water to survive. Perennial ryegrass is one of the most important forage grasses in Northern climate, but it is drought susceptible. The idea is to breed perennial ryegrass plants with improved architecture of root systems, which can better exploit available, deeper soil water, while maintaining above ground biomass yield. Some genotypes from a domestic perennial ryegrass cultivar showed a better performance in production and persistency under drought conditions. These genotypes showed large variability for root mass and distribution, deeper roots and better performance of root systems overall. Hence, the objectives of this study were to determine root depth distribution and dry matter production within an improved *Lolium perenne* population and to investigate changes in root architecture under different reduced water availability regimes. This trial was conducted the Institute for forage crops in 0.9m long plastic root-screening tubes filled with mortar sand. Individual plants of both populations (breeding and basic) were clonally divided into a minimum of eight small parts (ramets), each one with three tillers. The breeding population was selected in two cycles from the Serbian cultivar K11 (basic population), based on a better root architecture. The experiment was designed as a two factorial (genotype and irrigation level, 2x4) with three repetitions. Irrigation was initially performed by 100mlday^{-1} of low strength complete nutrient solution through a pipe system. After one month of growth, irrigation reduction was gradually started to maximum water content reduction (25%, 50%, 75%). The shoots were trimmed, air-dried and weighted. The roots were extracted from sand after four months of growth, cut into segments of 10cm in length, and then air-dried. The data were analyzed by standard ANOVA.

Number of plants with deep root fractions in the breeding population was higher in all watering regimes than in the basic population. The proportion of plants with roots below 90cm was 60% in maximum water reduction in the breeding population. Percentage of plants with deep roots was almost doubled with irrigation reduction (from 37.5 to 60%). Average dry matter yield of the breeding population was 11% higher in comparison with the basic population. The same time, average whole root dry mass was 7.7% higher, while dry mass of roots below 90cm was 20% higher. Nevertheless, dry mass of all categories of root was almost equal in both populations in maximum water reduction. But if we select from populations plants with the best DMY, percent of improvement of DMY rises to 18% and whole root dry mass to 21%. In this trial, correlation between DMY and whole root dry mass was statistically significant (0.71).

Breeding maize for resistance to Fusarium ear rot: impact of plant morphology for disease development and deoxynivalenol formation

Elzbieta Czembor[a], Seweryn Frasinski[a], Krzysztof Wojcik[b] and Jozef Adamczyk[c]

[a] *Forage Grasses and Legumes Dept., Plant Breeding and Acclimatization Institute-NRI, Poland*
[b] *Plant Breeding Smolice Ltd, Poland.*
[c] *Małopolska Plant Breeding Raising HBP Ltd, Poland*

e.czembor@ihar.edu.pl

Red and pink ear rots caused by *Fusarium* spp. are important factors affecting the yield and its quality, mainly because of contamination with mycotoxins produced by the fungi. In Poland, ear rot is commonly caused by *F. graminearum* producing deoxynivalenol (DON) and zearalenone and by *F. verticillioides* which produces fumonisins. It was observed during the last years, that contamination of grain by these toxins increase also in Poland. The development of resistant host genotypes strongly depends on availability of sources of resistance and information on host pathogen interactions. The resistance of maize to ear rots is very complex and depends on several components such as resistance to initial infection, resistance to fungal degradation of silk tissues, resistance to fungal spread by through a wax layer in the grain or grain morphology and chemical compounds of the pericarp. The accumulation of toxins can also be affected by the plant genotype. Although selection is effective to reduce disease severity after inoculation with *F. graminearum*, additional genes seemed to affect grain DON concentration (i.e., ratios between DON concentration and disease severity (DON/DS) in grains depended on genotype), indicating that specific mechanisms are present in the plant affecting DON production by de fungus and additional genetic progress would be achieved by including grain DON concentration as a selection parameter.

The aim of this study was to determine which plant traits play an important role for red ear rot development and DON formation in grain and rachis. As plant material, 28 hybrids and their parental inbreed lines were used. A Field experiment was conducted in three replications. For each genotype, eight plants were inoculated with *F. graminearum* and 8 plants were used as a control in each replication. During silking time, ear morphology was described: cob length, silk length (separately, covered and not covered by husks), antocyanin content in silks. Because of different ear morphology, the kernel inoculation method was used (9 11 days after silking). Disease development was visually assessed at harvesting time using a scale from 1-7. DON content was evaluated separately in grain and cob (rachis) samples with RIDA®QUICK SCAN using immunochromatographic tests. Relationships between disease severity, DON contamination, anthocyanin content and ear morphology were calculated using Pearson correlations.

Based on the obtained results it was possible to conclude that DON content (both, in grain and cobs) strongly correlated with disease severity. Disease severity and DON contamination, negatively correlated with anthocyanin content in silks and positively correlated with the length of silks which were not covered and covered by husks. In samples collected from the most resistant genotypes DON content was low under natural infection and also after inoculation.

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Popcorn genetic resources and breeding in Turkey

Sekip Erdal[a], Ahmet Ozturk[a], Mehmet Pamukcu[a], Erkan Ozata[b] and Mehmet Cavit Sezer[c]

[a] *Bati Akdeniz Agricultural Research Institute/ Field Crops Department, Antalya, Turkey*
[b] *Black Sea Agricultural Research Institute/ Field Crops Department, Samsun, Turkey*
[c] *Maize Research Institute/Adapazarı, Turkey*

sekip.erdal@gthb.gov.tr

Popcorn (*Zea mays everta*) is one of the oldest forms of field maize and it was developed for higher popping volume from flint maize type. Popcorn is a very popular appetizer in Turkey and its cultivation and consumption is increasing. It is widely cultivated in Aegean, Mediterranean, Southeast Anatolia, and Marmara region of Turkey. Popcorn seed was supplied mainly by the public sector's varieties in the past. Currently both public and private sector's hybrids are produced by the growers. The primary concern in popcorn production is lack of enough high yielding and quality local popcorn varieties. According to the Variety Registration and Seed certification Center of Turkey sources, there are only a few popcorn hybrids in the production. Therefore, popcorn growers are often having difficulty finding sufficient seeds. A national big scale popcorn breeding was initiated by public, private sector and university partnership in order to develop and release high yielding popcorn hybrids. With the Project, it was targeted to develop local popcorn hybrids and inbreeds to meet high yield and quality variety need and increase national production. In order to develop new inbreeds, genotypes will be derived from populations and pedigree breeding procedures will be applied. During the studies field and greenhouse will be used for generations. General combining ability tests will be done by top crossing method and the trials will be carried out in different 4 locations representing regions of Turkey. On the other hand, to develop local new varieties in a near future, promising inbreds of public and private sector will be crossed to each other in the light of genetic distances and breeding performances. Experimental hybrids will be evaluate in 4 locations in the 2nd and 3rd years of the Project. In the present study, information on popcorn genetic resources such as developed populations, collections and inbred lines in Turkey was given and the studies of the current breeding projects that carried out by the public sector were evaluated.

Automated field phenotyping of early vigour and senescence progress in soybean and maize

Frank Liebisch, Simon Strahm, Lukas Müller, Norbert Kirchgessner, Achim Walter

Institute of Agricultural Sciences, Federal Institute of Technology Zürich (ETHZ), Switzerland

frank.liebisch@usys.ethz.ch

Today, digital phenotyping is widely used under controlled conditions and more and more phenotyping systems are developed for field applications. However, often number of sensors and of monitored plant traits are limited and their relationship to traditional scores as used for breeding are often unsatisfying.
In this poster we show results from the first season of data collected with a rope suspended carrier system (Spidercam®) holding multiple sensors which can be positioned over a wide range of crop species, individual plots or plants: the FIP system (Kirchgessner et al. 2015). We present results on the detection of early vigour in maize and soybean, two contrasting crop species, within a set of early to late ripening genotypes, respectively. With a digital near infrared camera and a spectrometer a set of digitally detected crop traits, such as leaf chlorophyll indicators, plant size and soil coverage was monitored. The best indicators for early vigour and senescence progress will be shown. However, pre-processing algorithms and trait indicators will need species specific adaptation and interpretation because of the different morphology of the plants and canopies (plant size, canopy structure) and the different field setup (row interspaces) depicted in maize and soybean.
Similar approaches might improve detection of other breeding related crop traits such as stay-green, onset of flowering and pest and disease resistance. By means of the rope suspended system a high temporal resolution of measurement points can be obtained while keeping effects on the canopy (contact) at minimum.

Kirchgessner N, Liebisch F, Hund A, Walter A: Field imaging platform (FIP) – an automated system for plant phenotyping in the field. In 21 Workshop Computer-Bildanalyse in der Landwirtschaft und 3 Workshop Unbemannte autonom fliegende Systeme (UAS) in der Landwirtschaft am 7 Mai 2015 Braunschweig; 2015.

Advantages of using haploid technologies in maize breeding

Valeriu Rotarenco, Georgeta Dicu, Daniel State and Stelian Fuia

Procera Genetics, Fundulea, Calarasi, Romania

valeriu.rotarenco@procera.ro

Development of specific genotypes with an ability to induce maternal haploids *in vivo*, haploid inducers, was a breakthrough in the implementation of haploid technologies in maize breeding and research (Coe, 1959). However, using such technologies in major breeding programs with a large number of haploids produced from a wide range of genotypes under different conditions has required a significant improvement of the inducers. New haploid-inducing lines PHI (Procera Haploid Inducers) were developed in 2010 in Fundulea, Romania. The frequency of haploid induction was remarkably improved in the PHI lines - up to 15-20%; it is almost twice as high as in the best initial inducer MHI (Chalyk, 1999). Additionally to the main grain color marker gene, *R1-nj*, the PHI lines possess a combination of two genes, *Pl1* and *B1*, which compose a light-independent anthocyanin marker allowing haploids to be identified at the stage of 4-day-old seedlings and among mature plants in any donor. Agronomic traits such as plant height, tassel size, kernel production and logging resistance were also improved in the PHI lines. Within the last decade, the doubled haploid (DH) technology has become an important tool for the production of inbred lines in maize breeding. We have managed to develop a simple and rather efficient DH technique adjusted to the local conditions: the seedling surviving rate ranges between 60 and 80%; the frequency of male fertility can reach 50%; and about 25% of plants, relating to the total number of plants, form kernels after self-pollination. Every season, we have been producing more than 300 DH lines since 2008. During these years, it was noticed that the DH lines maintain a high level of homozygosity which is a very important property for our hybrid breeding program. In haploid and doubled haploid plants, allelic gene interactions (dominance and overdominance) are lacking facilitating the identification of genotypes with favorable non-allelic gene effects, which may have a great importance for the improvement of synthetic (heterogeneous) populations – breeding initial material. We have proposed to use haploid plants in a recurrent selection scheme - Haploid Recurrent Selection (HRS) to improve two synthetic populations, SP and SA, for *per se* performance (Chalyk and Rotarenco, 1999). After five cycles of HRS, the grain-yield increase per cycle was more than 10% in each population, which is much higher in comparison with the other known recurrent selection schemes (2-4%) (Hallauer and Mirinda, 1988). One of the improved populations, SP, has already reached the grain - yield level of the local F_1 hybrids used in maize production - 10 t/h. Currently, this population is used as an initial material in the project with a purpose to develop high-yield inbred lines. Haploid technologies have a high potential both for speeding up the breeding process and improving its efficiency. In our opinion, haploidy deserves much more application in maize breeding and research.

Morphological and molecular identity testing of maize accessions collected in the same area at different time scale

Violeta B.Andjelkovic[a], Dragan R. Kovacevic, Snezana D. Mladenovic Drinic, Mirjana B.Srebric, Natalija B. Kravic, Vojka B. Babic, Mirjana Jankulovska[b], Sonja Ivanovska[b] and Dane T. Bosev[b]

[a] *Maize Research Institute Zemun Polje, Serbia*
[b] *Faculty of Agricultural Sciences and Food, R. Macedonia*

avioleta@mrizp.rs

In the second half of 20th century maize local landraces in the former Yugoslavia (Western Balkan region) were replaced by hybrid maize. Due to expansion of hybrid maize landraces were less and less used but growing awareness of their importance for future led to their organised collection. The first collecting missions started in early 60-es of XXth century. At that time approximately 1000 samples were collected and classified according to agro-morphological descriptor. In next twenty years, collection continued and the total number increased up to 2217 accessions of local landraces stored at Maize Research Institute gene bank. Out of that number, 222 maize samples were collected in the Republic of Macedonia. During 2014, new collection missions were organised in the eastern part of the Republic of Macedonia. According to collecting site, kernel colour and kernel type, 15 samples from Macedonia were chosen for the identification of possible duplicates with the accessions from the same area and kernel characteristics within our gene bank. Samples were planted in two replications and two locations for morphological comparison. Phonotypical characterisation was done according to IBPGR descriptors for maize genetic resources (2008). Comparison between pairs of samples is presented for following traits: plant height, ear height, number of leaves above ear, ear length, number of rows per ear, number of kernels per row, kernel type, kernel colour, and 1000 kernels weight. Based on molecular analysis by 12 SSR markers, all samples from Macedonia were different from accessions in our gene bank. According to both, morphological and molecular data, maize samples recently collected in Macedonia could be treated as new, additional accessions in landraces collection of Maize Research Institute.

Maize landraces as a source for increased content of tocopherol and β-carotene in inbred lines

Violeta B. Andjelkovic, Jelena Z. Mesarovic and Snezana D. Mladenovic Drinic

Maize Research Institute Zemun Polje, Serbia

avioleta@mrizp.rs

Tocopherols, which are vitamin E compounds, play an important role in maintaining human health. Compared with other cereals, maize grains contain high level of tocopherols. Maize is a carotenogenic plant and its carotenoids are classified into carotenes and xanthophylls. Among them the *β*-carotene has the highest activity and is considered important in breeding programs of biofortified crops. Changes in carotenoids content in the maize grain could be influence of genotype x environment interaction, or effect of existing relationship between the yellow or orange color of the endosperm and the presence of carotenoids. This work was performed to estimate differences in tocopherol (*α-, β+γ-, δ-)* and *β*-carotene content in three landraces (with orange, brown and dark red grain), five commercial inbreds and their crosses and reciprocal crosses, by high-performance liquid chromatography (HPLC). Significant phenotypic variation was observed among the genotypes in the traits of interest. Total tocopherol (*α-, β+γ-, δ-)* content was higher in all crosses with landraces of orange and red grain (16.13 to 53.27 μg/g), except in crosses with line L5, compared to lines *per se*. However, *α*-tocopherol content was higher in the crosses with orange grain landrace (1.97-7.99 μg/g), compared to lines (1.65-7.21 μg/g). In the crosses between lines and red grain landrace, *α*-tocopherol content varied (was lower or higher) compared to lines *per se*. Crosses of landrace with the brown grain had higher total tocopherol content then lines *per se*, except the lines L4 and L5, but *α*-tocopherol content was higher in all five tested lines compared to their crosses. *β*-carotene content in inbreds vary from 0.47 to 2.98 μg/g, and in crosses the highest content were in progeny from landrace with orange grain, ranging from 3.22 to 16.48 μg/g, although the lowest variation was in crosses by landrace with red grain, from 0.25 to 7.6 μg/g. Landrace with orange grain could be used in future breeding programs for the improvement of nutritive value of commercial inbred lines, and landraces with brown and red grain will be tested for antioxidative activity and anthocyanin content.

Molecular indicators of resistance to *Microdochium nivale* in *Lolium multiflorum/Festuca arundinacea* introgression forms

Adam Augustyniak[a], **Katarzyna Masajada[a]**, Agnieszka Płażek[b], Ewa Pociecha[b], Michał Dziurka[c] and Arkadiusz Kosmala[a]

[a] *Institute of Plant Genetics of the Polish Academy of Sciences, Poland*
[b] *Department of Plant Physiology, University of Agriculture in Cracow, Poland*
[c] *The Franciszek Górski Institute of Plant Physiology of the Polish Academy of Sciences, Poland*

kmas@igr.poznan.pl

Lolium multiflorum (Italian ryegrass) possesses high forage quality but low tolerance to abiotic and biotic stresses. *Festuca arundinacea* (tall fescue) expresses higher winter-hardiness, drought tolerance and resistance to diseases. The intergeneric hybrids of both species and their introgression derivatives combine those complementary attributes. In the research presented here, the BC_5 *L. multiflorum/F. arundinacea* introgression forms were evaluated with respect to their level of resistance to *Microdochium nivale*. Among 20 *L. multiflorum/F. arundinacea* forms a wide range of diversity with respect to this trait was observed. Four individuals were selected for further molecular research - two (180/30/19 and 180/30/75) with a high level of resistance and the other two (180/30/84 and 180/30/138) with no resistance to *M. nivale*.

Leaves and crown tissues of the selected introgression forms were analyzed with respect to soluble carbohydrate, phenolic compounds and abscisic acid contents after pre-hardening and hardening periods, and also one and seven days after inoculation. The performed analysis revealed that *M. nivale* increased significantly soluble carbohydrate content in both, leaves and crown tissues of all the investigated introgression forms. However, the accumulation level of these primary metabolites was much higher in the resistant individuals. A similar accumulation pattern was also observed for phenolic compounds. On the other hand, the accumulation level of abscisic acid was shown to be much lower seven days after inoculation in the analyzed organs of resistant forms, compared to the susceptible forms. The role of the analyzed compounds in the mechanisms of grass resistance to *M. nivale* is discussed.

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UPOV morphological versus molecular markers for maize inbred lines variability determination

Vojka Babić[a], Ana Nikolić[a], Violeta Andjelković[a], Dragan Kovačević[a], Milomir Filipović[a], Vladimir Vasić[b] and Snežana Drinić Mladenović[a]

[a]*Breeding Department,Maize Research Institute Zemun Polje, Serbia*
[b]*Department of statistics, Faculty of Economics, University of Belgrade, Serbia*

vbabic@mrizp.rs

In hybrid breeding programmes, it is very important to define the genetic distance of inbreds belonging to the same or different heterotic groups. In addition, it is important to define criteria and biometric methods for the satisfactory germplasm classification. Despite the significant progress made by the development of molecular techniques, there are still certain difficulties that have to be overcome. Firstly, the type of markers and adequate coverage of the genome have to be defined. Even within the same species, there are indications that different germplasm pools require a different number of markers for adequate genetic divergence evaluation. Furthermore, it seems that gene epistatic effects may play a significant role in final defining of the phenotype and thereby of its agronomic traits. Morphological markers are considered, by many authors as unreliable indicators of genetic relationships, but a certain extent, the existing inconsistency between molecular and morphological data could be the result of an incorrect methodological approach in gathering morphological data, as well as, of an inadequate choice of type and number of molecular markers used. Also, due to an insufficiently clarified phenomenon of heterosis, field tests are still the most time and resources consuming.

This research describes how to use phenotypic characterisation, according to the UPOV descriptor, for getting useful information in maize breeding. The comparisons of morphological and molecular markers were also performed. Results indicate that the application of the UPOV descriptors in phenotypic characterization, as well as use of adequate statistical methods and scale of measurements, increases the quality of obtained information. Based on visual assessment of plant groups (MVG), morphological markers acceptably discriminated and grouped tested lines. It should be emphasized that the phenotypic characterization by visual assessment of a group of plants or parts of plants, according to UPOV descriptor, is simple, does not require great investments and is not labor consuming. Unfortunately, information obtained by a small number of both SSR and RAPD markers, did not give significantly better results. For obtaining more reliable information, the number of molecular markers needs to be significantly higher.

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Evaluating agronomic performance and investigating molecular structure of drought and heat tolerant wild alfalfa (*Medicago sativa* L.) collection from the Southeastern Turkey

Mehmet Basbag[a], Ali Aydin[a] and **Muhammet Sakiroglu[b]**

[a] *Department of Field Crops, Dicle University, Turkey*
[b] *Department of Bioengineering, Kafkas University, Turkey*

msakiroglu@kafkas.edu.tr

Drought is a major stress factor for agricultural production including alfalfa production. One way to counterbalance the yield losses is the introgression of drought tolerant germplasm into breeding programs. As an effort to exploit such germplasm, 16 individual plants were selected in their natural habitat in Southeastern Turkey and clonally propagated in field trials with an ultimate goal to use the germplasm as parents for releasing a synthetic cultivar. Forage yield and forage quality traits were evaluated and molecular genetic diversity among genotypes were determined using ISSR markers. Genotypes showed a variation from growth habit to yield and quality traits indicating sufficient phenotypic variation for diverse breeding efforts (for grazing or harvesting) and long term selection schemes. A large amount of genetic variation was observed even with a limited number of markers and genotypes. However, no spatial pattern of genetic structure was observed when genetic distance was compared to the geographic distance. We conclude that *ex situ* natural variation provides a wealth of germplasm that could be incorporated into breeding programs aiming to improve drought tolerance. We also suggest an extensive collection of seeds/plant tissue from unique plants with desirable traits rather than putting more efforts to create a spatial germplasm sampling efforts in narrow regions.

Identification of forage grass germplasm for water-limited environments and different soil types

Elzbieta Czembor [a], Seweryn Frasiński [a], Ryszard Golimowski [b], Jan Schmidt[b], Włodzimierz Majtkowski [b]

[a] *Forage Grasses and Legumes Dept., Plant Breeding and Acclimatization Institute-NRI, Poland*
[b] *Botanical Garden, Plant Breeding and Acclimatization Institute-NRI, Poland*

e.czembor@ihar.edu.pl

Permanent grassland are a source of healthy forage for a large group of ruminant animals. They also serve to conserve biodiversity, reduce environmental pollution, including nitrogen oxide and sulfur in the air. They can contribute to the agro ecosystem sustainability by reducing soil erosion and conserving soil water. Ecotypes may provide genetic resources to improve resistance / tolerance for water stress or different soil types. Water conservation is the responsibility of every citizen, not just in areas with drought or low moisture conditions. Drought resistance is being increasingly labelled as being a 'complex trait'. Preliminary tests could be conducted under controlled conditions, however results should be confirmed under field conditions.

In the present study, ecotypes and commercial hybrids which belong to 7 cool-season grass species were used: tall fescue (*Festuca arundinacea*), meadow fescue (*F. pratensis* Huds.), red fescue (*F. rubra* L.), perennial ryegrass (*Lolium perenne* L.), Timothy-grass (*Phleum pratense*), Kentucky bluegrass (*Poa pratensis*) and *Deschampsia cespitosa*. Ecotypes were collected from semi-natural areas representing different parts of Poland. Based on the preliminary description, 15 – 17 genotypes from each species, were included in this study. Plants were evaluated under field conditions (seed production system – one environment and green mass production system – second environment) and under greenhouse conditions (drought tolerance and soil type tolerance).

For the greenhouse test, seedlings were taken from the experiment conducted under field conditions and after vegetative propagation they were planted into pots. They grew for six weeks at an optimum moisture content of the soil (35 – 42%; soil type - mixture: 3 peat : 1 sand), were cut every 7 days at a height of 7 cm (red fescue - 4 cm), and regrowth was measured. After this, drought resistance and tolerance to different soil types was evaluated: three soil types (control with 3:1 peat:sand and two soils from devastated areas) and two water treatments (watered control and drought stress for 4 weeks with 8-10% soil moisture) were used. It was found that: (1) timothy, and red fescue are characterized by the larges decrease in the rate of regrowth under water defiicit, (2), tall fescue and *Deschampsia cespitosa* quickly regenerate after a period of water deficit (3) meadow fescue has a low ability to regenerate after water deficit, and (4) in all species it was possible to find genotypes tolerant to water deficit.

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Determination of second crop silage maize cultivars for no-till conditions

Huseyin Dulkar [a], **Taner Akar** [b] and Mahmut Kaplan[c]

[a] *Erciyes University, Institute of Natural and Applied Sciences, Turkey*
[b] *Akdeniz University , Faculty of Agriculture, Antalya, Turkey*
[c] *Erciyes University, Faculty of Agriculture, Kayseri, Turkey*

tanerakar@akdeniz.edu.tr

This research was carried out to determine the second crop silage maize cultivars suitable for no-till conditions after cereal +vetch mixtures by using 22 hybrid maize cultivars under randomized complete block design with three replications during the 2013 cultivation season in Kayseri province. In this research, the highest green herbage yield, plant height and cob length were obtained by ADA-9516 (98,4 t/ha), ADA-9516 (2,83 m) and BOLSON (31.00 cm) cultivars, respectively. Moreover, the highest dry matter, the more digestible ADF and NDF rates were determined by KOPIAS (26.82 %), BOLSON (25.13 %) and KERBANIS (43.71 %) cultivars, respectively. Under no-till conditions, top silage maize cultivars for herbage yield, ADF and NDF were ADA-9516, BOLSON and KERBANIS respectively. According to this research, cultivars ADA-9516, DKC 6903, TRUVA and CADIZ are suggested for the highest herbage yield while cultivars BOLSON, KOMPOZİT ARİFİYE, PR 31 Y 43 and ADA 9516 for the highest silage quality under no-till conditions for the second crop silage maize production in Kayseri province.

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Determination of second crop silage maize cultivars for reduced tillage conditions

Songul Han [a], **Taner Akar** [b], Mahmut Kaplan[c]

[a] *Erciyes University, Institute of Natural and Applied Sciences, Turkey*
[b] *Akdeniz University , Faculty of Agriculture, Antalya, Turkey*
[c] *Erciyes University, Faculty of Agriculture, Kayseri, Turkey*

tanerakar@akdeniz.edu.tr

The aim of this research was to determine effects of reduced tillage conditions to herbage yield and quality of second crop silage hybrid maize cultivars. For this reason, 22 hybrid maize cultivars were planted under randomized complete block design with three replications during the 2013 cultivation season in Kayseri province. In this research, some parameters were investigated from green herbage to neutral detergent fiber (NDF) and the highest green herbage yield, plant height and cob length were obtained by ADA 8924 (85,4 t/ha); Truva (3.03 m); and Prisca (31.61 cm) cultivars, respectively. In addition to these, the highest dry matter, and the more digestible ADF and NDF rates were gathered by Bolson (25.55 %) ; Prisca (23.67 %); and Prisca (41.33 %) cultivars, respectively. Under reduced conditions, top silage maize cultivars for herbage yield, ADF and NDF were ADA 8924,; Prisca; and Prisca respectively. According to this research, cultivars ADA 8924, 30B74, Samada, and Hido are suggested for the highest herbage yield while cultivars Prisca, Bolson, ADV 2898, and Kerbanis for the highest silage quality under reduced tillage conditions for the second crop silage cultivation in Kayseri.

Acknowledgements : financial contribution of the University Erciyes is acknowledged.

Variation in water use efficiency among maize inbred lines with different drought tolerance

Natalija Kravic[a], Vesna Dragicevic[a], Violeta Andjelkovic[a], Vojka Babic[a], Danijela Ristic[a] and **Jelena Srdic[a]**

[a]Maize Research Institute Zemun Polje, Serbia

jsrdic@mrizp.rs

Water scarcity, further worsened by climate changes, is one of the major constraints to the world's demand for food production. Besides improved agronomic and water management practices, improvements in grain yield and crop water productivity arise from breeding for superior varieties. The natural, semiarid environment, toward which breeding work is often directed, is rather difficult for plant selection, because of very high intra- and inter-seasonal variations in timing and amount of rainfall received. The key to improve rate of progress in breeding and selection has been the use of managed field-based stress around the flowering period. For this purpose, two-year of screening for drought tolerance under control drought in Egypt, and further testing in the temperate climate regions of Macedonia and Serbia, were conducted in period from 2007 to 2010. As a result, a mini-*core* collection of 41 accessions (fifteen maize inbred lines and twenty-six landraces) was established. Drought tolerant inbreds from Maize Research Institute mini-*core* collection (from DTL1 to DTL15) and maize public lines B73 and A632 were tested in field experiments without irrigation, conducted in 2014 and 2015, at Zemun Polje (44°52´N, 20°19´E, 81 m asl). The soil was slightly calcareous chernozem with 47% clay and received the usual compound of mineral fertilizer. Genotypes were tested in two-replicate trials, set-up using Randomized Complete Block Design, in two plant densities. Moisture content in soil was determined gravimetrically. Soil samples were taken at sowing and at harvest, from eleven different layers, on 0-200 cm soil profile. Apparent water use during the growing period (April-September), expressed as evapotranspiration, was calculated from seasonal rainfall and soil water consumption data during the growing period. Grain yield was determined at harvest, and water use efficiency (WUE) was calculated from ratio between grain yield and evapotranspiration. In dry 2015, ten DT inbreds exhibited average increase in WUE of 46.1%, ranging from 19.6% (DTL14) to 80.0% (DTL9), compared to 2014 as optimal year for maize growth and production; with seven of them achieving 16.8% of WUE increase, in higher plant density. In both plant densities, average WUE decrease of 61.2% was recorded in two tested public lines. In this experiment, highly significant and positive correlation between % of change in WUE and grain yield ($r=0.676$; $P\leq0.01$) was found. Based on the results, increased ability in water use efficiency under water deficit conditions, followed by reduced decline in grain yield, was found in DTL1, DTL7, DTL9, DTL11 and DTL12. Those inbreds could be considered and recommended as a favorable source for drought tolerance in breeding programs.

Characterisation of maize inbred lines of unknown pedigrees or heterotic groups for pre-breeding

Sanja Mikić[a], **Ankica Kondić Špika[a],** Dušan Stanisavljević[a], Ljiljana Brbaklić[b], Bojan Mitrović[a], Dragana Trkulja[a] and Marina Tomičić[a]

[a]*Institute of Field and Vegetable Crops, Novi Sad, Serbia*
[b]*Research and Development Center Biogranum, Novi Sad, Serbia*

ankica.spika@ifvcns.ns.ac.rs

The introduction of new germplasm into existing breeding programmes is an essential step for broadening genetic diversity of working breeding material and could serve as an untapped source of favourable alleles. Maize breeders at the Institute of Field and Vegetable Crops enrich their collections with adapted exotic germplasm, introduced accessions from gene banks and old local landraces and inbred lines developed from them. However, such genetic material often has uncomplete pedigree information and unknown heterotic response. Traditionally, in such cases, crosses with inbred lines of different known heterotic groups are made and their F_1 offspring is observed for hybrid vigour, requiring two seasons and considerable land and labour resources. In order to utilise maize inbred lines developed from less-known introduced germplasm for breeding purposes in less time- and labour-consuming way, we analysed and compared molecular and phenotypic data of previously uncharacterised inbred lines to those which pedigrees and heterotic groups are well-known. Based on 30 microsatellite marker data, a principal coordinate analysis was performed to group the inbred lines into four clusters: Lancaster, BSSS, Iodent and unrelated independent heterotic groups. Slightly different grouping was obtained with phenotypic morphological data used in principal component analysis. The field validation of both methods showed that the use of molecular markers is more efficient in assigning inbred lines to their corresponding heterotic groups and consequently planning crosses. Our results demonstrate that a pre-breeding inbred line characterisation could be achieved with satisfactorily accuracy using fewer markers.

Acknowledgement
To the Serbian Ministry of Education, Science and Technological Development under the project TR31073 for the research funding.

Assessment of performance of early-maturing white maize hybrids and testing sites using GGE biplot analysis

Muhyideen Oyekunle[a], Alidu Haruna[b], Baffour Badu-Apraku[c], Inuwa S. Usman[a], Hamza Mani[a], Shehu G. Ado[a], Gbadebo Olaoye[d], **Kwadwo Obeng-antwi[b] and Rekiya O. Abdulmalik[a]**

[a]Institute for Agricultural Research, Ahmadu Bello University, Nigeria
[b]Savanna Agricultural Research Institute, Ghana
[c]International Institute of Tropical Agriculture, United Kingdom
[d]University of Ilorin, Nigeria
[e]Crops Research Institute, Ghana

rekimalik83@yahoo.com

Identification of outstanding maize hybrids for target environments is complicated by genotype × environment interactions. Thirty-six early-maturing white-endosperm maize hybrids were evaluated at 14 locations in Nigeria and Ghana for 2 years. The objectives were to (i) identify superior and stable hybrids across environments, (ii) classify the test sites into mega-environments, and (iii) identify core testing sites for selection of superior maize hybrids in the two countries. Genotype, environment and genotype x environment interactions were significant (P<0·01) for grain yield and most other traits. Grain yield of the hybrids ranged from 3177 kg ha^{-1} for EWH-5 to 4596 kg ha^{-1} for EWH-29. The GGE biplot analysis revealed that EWH-29 and EWH-30 were the highest yielding and most stable hybrids while EWH-4 and EWH-5 were the lowest yielding but stable across environments. Hybrids EWH-37 and EWH-40 were high yielding but unstable. Test sites were classified into three mega-environments: Samaru and Ilorin constitute the first group, the second group consists of Basari, Kafin Soli, Talata Mafara, Mokwa, Lapai, Ejura, and Pokuasi while the third group comprises Nyankpala, Fumesua, Manga, Minjibir, and Damongo. Samaru and Ilorin were highly correlated in their ranking of the hybrids; however, Samaru was more discriminating and thus, identified as the core test site for group 1. Similarly, the five locations in group 2 were highly correlated and Kafin Soli was identified as the core test site. Minjibir had the highest discriminating power in group 3 and thus, identified as core test site. EWH-35, EWH-37, and EWH-34 were the vertex hybrids in mega-environments 1, 2 and 3, respectively. Samaru, Kafin Soli and Minjibir were identified as the core testing sites for identification of superior maize hybrids for the two counties. The identified hybrids should be commercialized to contribute to alleviation food insecurity and poverty in Nigeria and Ghana.

A breeding value assessment of Bulgarian maize local varieties

Albena Pencheva[a] and **Stefan Vulchinkov[b]**

[a]*Institute for Plant Genetics Resources "K. Markov"- Sadovo, Bulgaria*
[b]*Maize Research Institute – Kneja, Bulgaria*

stefan_vulchinkov@abv.bg

A set of 44 maize local varieties (populations, FAO 300-400) from the collection of IPGR after pre-breeding were crossed with 3 testers from different heterotic groups – 118/96B (Lancaster), PHK-42 (Iodent), DK 16-G_2 (unknown). The field trials were carried out in Sadovo under irrigation (2012-2015). Big scale data were collected and studied, including quantitative traits: grain yield and its components, grain quality, events of heterosis . ANOVA with Duncan`s test and cluster analysis were applied.

A few testcrosses – 3 with 118/96B and 4 with DK 16-G_2 exceeded significantly grain yield of middle early checks – Kn 435 and Lg 3475, and no ones PR 9578, but many of them surpassed the same checks by crude protein and oil content in the grain. As a general trend they had 10-12% crude protein (average 10.75%), compared to checks – 9.07% and less (70.77% to 77.70%) starch content. Average oil content was 3.66% for checks and 4.80% for all test-crosses. Many cases were observed with high values of protein, oil and starch content individually, but one test cross (17) combined high values for all three parameters and separated a single cluster (fig. 1). The assessment of these local populations – "per se" and top cross – pointed out valuable sources of diversity for selection programs, especially breeding of quality.

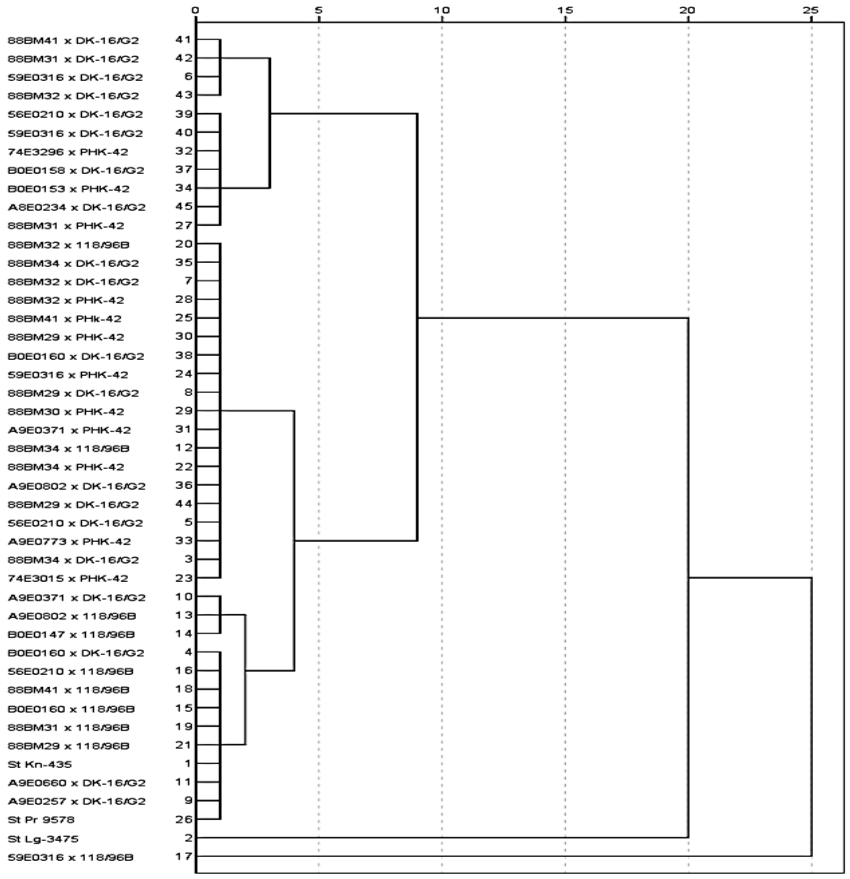

Fig.1 Clustering of testcrosses about grain quality content

Using genome wide allele frequency fingerprints to identify allele frequency changes in seeded perennial ryegrass swards

Aude Perdereau[a], Dan Milbourne[a], Stephen L. Byrne[a] and Susanne Barth[a]

[a] *Crops, Environment & Land Use Programme, Teagasc, Oak Park, Carlow, Ireland*

aude.perdereau@teagasc.ie

Unlike varieties of many crops, in which all plants of a variety are identical, individual perennial ryegrass (PRG) cultivars are effectively genetically divergent populations of individuals that have been selected to have broadly similar characteristics. In addition, as opposed to annual crops, PRG cultivars are expected to remain in the field over a near decadal period before re-seeding. One consequence of these factors is that, once planted, the composition of perennial ryegrass swards can change over time in response to environmental variation and management practice. It was estimated that only 10% of the PRG plants sown in a sward survive after 10 years [1].

In order to gain a better insight into this phenomenon, we will use cutting edge genetic fingerprinting approach called "Genome Wide Allele Frequency Fingerprints" (GWAFFs, based on a genome-wide marker approach called genotyping by sequencing [2, 3]) to monitor changes in the genetic composition of PRG swards over time. We will test whether these changes are random, or whether they can be related to management practices causing some plants to be preferentially selected over others. The ultimate goal of the research is to identify regions of the genome that are responsible for determining the lifetime performance characteristics of grazed swards and to use this information in the future to develop better performing PRG cultivars using genomic selection-based strategies similar to those currently used in cattle breeding.

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Development of dual use maize cultivars for grain and biogas production

Luisa Pfalsdorf[1], Walter Schmidt[2] and Heiko C. Becker[1]

[1] *Department of Crop Science, Georg-August Universität Göttingen, Germany*
[2] *KWS SAAT SE, Germany*

luisa.pfalsdorf@agr.uni-goettingen.de

Land resources for the production of food, feed and energy are limited. Therefore dual use maize varieties will be developed combining the use of kernels for feeding animals with the use of leafs and stem as substrate for biogas production. This will reduce the conflict between producing food and feed or producing energy.
Dual use maize varieties have to combine characteristics different from kernel maize, silage maize, or energy maize:

1. High grain yield (nearly as high as kernel maize)
2. High yield of rest plant (stem and leaves)
3. High water content of rest plant
4. High sugar content of rest plant
5. High methane yield of rest plant

The project is running for three years. In the first year (2014), strategies and methods to measure sugar content, photosynthetic activity and yield have been investigated. In multilocational trails 180 testcrosses were grown, the best lines were selected and have been crossed in a winter nursery by KWS. In the second year (2015), the new hybrids were tested for yield, sugar content and photosynthetic activity. In the third year (2016) it is planned to identify molecular markers for sugar content and photosynthetic activity.
A high genotypic variability for sugar content and photosynthetic activity of the rest plant was observed and these traits have a high heritability. It not easy to combine high grain yield with high yield of the rest plant, but high performing dual use hybrids could be selected and will soon be tested in the official performance trials for cultivar release.

Characterisation and further evaluation of local maize landraces within prebreeding activities

Aleksandar Popović [a], Slaven Prodanović,[b] Natalija Kravić [a], Mile Sečanski [a], Jelena Golijan [b], and **Vojka Babić** [a]

[a]*Maize Research Institute Zemun Polje, Breeding Department, Serbia*
[b]*Faculty of Agriculture, University of Belgrade, Serbia*

vbabic@mrizp.rs

Although considered as a valuable source for breeding purposes, local maize landraces represent a raw material loaded with unfavourable agronomic traits. Prebreeding is the most efficient method to overcome the existing gap between genetic resources and the process of commercial breeding. Previously selected under controlled drought conditions in Egypt, 310 local landraces from Maize Research Institute Zemun Polje (MRIZP) Gene Bank were subjected to further characterization. Based on 27 morphological traits, along with the application of hierarchical cluster and discriminant analyses, 11 groups were created. During the further evaluation, 31 best performing local landraces that exhibited small values for ASI and ear height, high yield *per se*, dark green leaves, small number of lodged and broken plants and high quality of ears were selected, with the emphasis on early maturity flint landraces with cylindrical ears. The same landraces were selected for further testing of their heterotic potential, as a key factor for utilising maize germplasm. For this reason, landraces were crossed with three divergent inbred testers from Lancaster, BSSS and Independent source. *Top-cross* hybrids produced, are going to be tested in two-year of multilocational trials (in 2015 and 2016), set up with the aim to define heterotic pattern of the selected landraces. Moreover, genetic characterisation of landraces by application of molecular markers will be included. Combining of all information to be obtained, there are two ways of using selected landraces: formation of broad based composites, from the landraces with similar morphology and heterotic pattern. Faster way for improving selected landraces is application of backcrossing method with related elite inbreds (through one or two backcrosses). Combination of these two approaches provides probably the best balance in achieving the short, medium and long term breeding goals. Developed synthetic populations will broaden the genetic base of MRIZP breeding material.

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Investigating the genetics of self-fertility in an F2 population of *Lolium perenne* L.

Lucy M. Slatter and Daniel Thorogood

Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, United Kingdom

dnt@aber.ac.uk

The economically important grass species *Lolium perenne* L. and other Poaceae species have gametophytic self-incompatibility (SI). Self-incompatibility in *L.perenne* is controlled by two genetic loci; S and Z. However, self-incompatibility in *L.perenne* does not always fully prevent self-fertilisation. Only one additional locus, T, has previously been found to influence self-compatibility, indicating that SI in the grasses is multigenic. The exact mechanism controlling self-incompatibility is still unknown, hence further research is required. To investigate the genetics of self-fertility in *L.perenne* a series of *in vitro* self-pollinations were conducted on an F2 population. Quantitative trait loci (QTL) analysis revealed a significant association between single nucleotide polymorphism markers and self-compatibility on linkage group 6 (LG6) of *L.perenne.* Therefore a previously undescribed self-compatibility locus has been found on LG6 in *L.perenne.* This finding was supported by significant segregation distortion coinciding with the QTL region. Further investigation of the markers making up the QTL found a region containing 179 *Brachypodium distachyon* L. genes underlining the QTL. Based on literature, candidate genes of interest were suggested, particularly relating to the potential involvement of kinases and monogalactosyldiacylglycerol synthase genes. Due to *L.perenne's* syntenic relationship to other Poaceae species including those of high agronomic importance, such as *Triticum aestivum* L., information about self-fertility can be transferred using comparative genomics. This could potentially allow breeders to exploit hybrid vigour in breeding systems to produce superior lines of *L.perenne* and other species. Overall this finding could improve crop development in a globally changing environment.

Agronomic characteristics and chemical composition of ZP sweet corn hybrids

Jelena Srdić[a], Zorica Pajić[a], Marija Milašinović Šeremešić[a], Milica Radosavljević[a], Valentina Semenčenko[a], Vojka Babić[a], and Natalija Kravić[a]

[a]*Maize Research Institute Zemun Polje, Serbia*

jsrdic@mrizp.rs

Sweet corn is very popular vegetable worldwide. It is consumed in the milky stage of endosperm development, fresh and processed. It is an important source of fiber, minerals, proteins and certain vitamins. Eating quality of sweet corn mostly depends on the sugar content in the endosperm primarily sucrose. Beside sucrose, sugars such as glucose, fructose and maltose contribute the sweetness of sweet corn. The recessive *su* allel in normal sweet corn modifies flavor, tenderness and texture of kernel and on the other hand, agronomic characteristics of sweet corn hybrids such as appearance of plant, ear and kernel. The effect of this allele is that, in the milky stage of endosperm development in sweet corn, there are nearly three times more reducing sugars and sucrose, ten times more water soluble polysaccharides and three times less starch content compared to standard quality corn. All of this produces the recognizable taste, texture and aroma of sweet corn. In sweet corn breeding programs, the yield is one of the most important traits, but the modes of consumption, morphological traits of the plant, ear and kernel and kernel chemical composition are also very important. Favorable traits of sweet corn hybrids are uniformity of ear size, length and shape, proper kernel row configuration, depth, width and color of kernels, as well as quality of the taste provided by pericarp tenderness, sweetness and creamy texture given by the chemical composition of the endosperm. In this paper we analyzed some agronomic and chemical quality parameters of 12 sweet corn hybrids developed at Maize Research Institute Zemun Polje, Serbia. Harvest was performed 23 days after pollination i.e. silking. Average fresh ear yield of twelve sweet corn hybrids was 12.46 t/ha, and it ranged from 10.68 for ZP 424su to 13.95 t/ha for ZP 446/1su. The number of kernel rows ranged from 14 to 20. Dry matter content at harvest ranged from 20.1 to 26.0%. Chemical composition of twelve sweet corn hybrids was determined according to the content of non fiber carbohydrate (NFC), total proteins, oil, crude cellulose, neutral detergent fiber (NDF) and ash. Also, protein fractions, i.e. albumins, globulins, α-zein and glutens were determined. The content of NFC ranged from 69.64% to 77.29%, while the content of total proteins did not vary much among hybrids, ranging from 10.26% to 11.98%. The variation in oil content among hybrids was noticeable (3.81 – 6.89%). Among protein fractions, the most abundant were albumins (29.42%), followed by α-zein (21.11%) and glutens (19.77%), while the lowest content was found in globulins fraction (5.64%).

Molecular characterisation of *Fusarium graminearum* chemotypes originated from Serbia

Slavica Stanković[a], Ana Obradović[a], Dragana Ignjatović Micić[a], Ana Nikolić[a], Jelena Stepanović[b] and Bojan Duduk[b]

[a] *Maize Research Institute Zemun Polje, Serbia*
[b] *Institute of Pesticidesand Enviromental Protection, Zemun, Serbia*

sstojkov@mrizp.rs

Members of the *Fusarium graminearum* species complex (FGSC) are important plant pathogens, they cause Fusarium head blight or scab of wheat, barley and rice, and ear rot of maize. In addition to quantitative yield losses, harvested grain sustains qualitative problems of contamination with mycotoxins. At least 13 species produces mycotoxins which are a serious threat to human and animal health. One of the most significant mycotoxin groups are trichothecenes. Three chemotypes can be distinguished within FGSC populations: NIV chemotype for strains producing NIV and 4NIV; 3ADON chemotype for strains producing DON and 3ADON; 15ADON for strains producing DON and 15ADON. The objective of the study was to determine trichothecene production profiles in tested samples, using PCR analysis. A total of 24 *Fusarium graminearum* isolates originated from grain samples collected from wheat, maize and barley kernels in 20 localities in Serbia were analysed. Genomic DNA was isolated and amplified with PCR using specific primers for TRI3 and TRI12 genes. TRI3 gene analysis showed presence of the 15ADON chemotype in all samples, the same as in TRI12 gene analysis. PCR tests present a direct and fast method for the trichothecene chemotype determination within different *Fusarium graminearum* species complex isolates and can be incorporated in programs for monitoring occurence, distribution and impact of these mycotoxins in different areas and species.

Improving biodiversity in energy crop production by mixed cropping of maize and climbing beans

Mathias Starke [1], Walter Schmidt [2] and Heiko C. Becker [1]

[1)] *Department of Crop Sciences, Georg-August-Universität Göttingen, Germany*
[2)] *KWS SAAT SE, Germany*

mathias.starke@agr.uni-goettingen.de

Maize is of paramount importance as substrate for biogas plants in Germany. However, the increasing cultivation of maize can also be regarded as problematic for sustainable agriculture. An interesting alternative to sole cropping of maize is its mixed cropping together with legumes, especially with climbing beans (*Phaseolus vulgaris*). Without losing the high yield potential of maize, biodiversity could be enhanced and soil fertility could be increased.
In previous years maize varieties with high yield potentials combined with an adaption to mixed cropping with climbing beans were successfully selected. During these studies it became clear however, that also the selection of adapted climbing bean varieties is necessary to attain high total yields.
For this purpose, 12 pre-selected climbing bean varieties were tested in field experiments for their suitability to the mixed cropping system with 8 energy maize varieties in three locations. The focus is on a late maturity and high biomass production of the beans.
In addition, we conducted a germination test under suboptimal temperatures with 200 commercial bean varieties as well as a freezing test of their seedlings in a climatic chamber.
Performance tests in 2015 showed, that total yields of specific combinations of maize and bean varieties are able to keep up with sole maize yield. Furthermore there is a high diversity among bean varieties in the ability of germination under low temperatures (8 to 12 °C) as well as in the ability to resist several nights of frost stress (-3 °C). These results demonstrate the possibility to sow beans and maize simultaneously in late April, which is crucial for a cost effective application of this mixed cropping system.

Assessment of the degree of relatedness of some inbred lines adapted to the early maize growing regions, created at ARDS Turda

Andrei Varga [a, b], Voichita V. Has[a], Roxana E. Calugar[a, b], Carmen D. Vana [a], Ana Copandean [a] and Ioan Has[a,b]

[a] *Agricultural Research and Development Station Turda, Romania*
[b] *University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania*

andrei_varga06@yahoo.com

Knowledge of relationships among elite breeding materials is useful in planning crosses for hybrid and line development, in assigning lines to heterotic groups, and in plant variety protection. It can be obtained from pedigree and heterosis data, from morphological traits or using molecular markers. The objectives of this study were to investigate phenotypic and genetic similarities/diversities between 7 flint (common origin), inbred lines adapted to the early maize growing regions, created at ARDS Turda-Romania. They were investigated in 28 crosses: 7 flint inbreds x 4 inbred lines testers (2 have the same origin as the 7 flint inbreds and 2 have a different origin). The inbred lines were crossed using a factorial mating design (m • n). Both parental inbreds and hybrids were evaluated for 22 traits in four replications and during two years (2013-2014) in Turda, Romania. The level of relatedness/ diversities were determined by various methods: pedigree, morphological characters, midparent heterosis (MPH), specific combining ability (SCA) and phenotypic and genotypic correlations between the lines of the same group. Morphological characters of the 11 inbred lines and 28 hybrids were performed according to the UPOV descriptor. From the point of view of phenotypic characteristics observed that the 7 flint lines (common origin) are not differentiated significantly from testers TB 329 and TC 177, for example, the line TD 234 is related to the tester TB 329 and is distinct from the other tester TC 177 (European flint, heterotic group) for certain characters such as: number of rows, number of kernels / row and 1000 grain weight.
The calculated heterosis for grain yield, ear weight and grain weight was very low for the hybrids obtained by crossing 6 of the 7 studied lines with tester TB 329. The lack of heterosis proved that studied lines are related to the tester TB 329 (Iodent, heterotic group). The purpose of this paper was the assessement of the degree of relationship between the lines created from a single source of starting material, which allowed the establishment of their identity, a necessary element in the selection and hybridise process.

A fifth teen-year tendency of productivity and grain moisture of Bulgarian maize hybrids by FAO groups

Vulchinkov S[a], P. Vulchinkova[a] and K. Petrova[a]

[a]*Maize Research Institute, Kneja, Bulgaria*

stefan_vulchinkov@abv.bg

Different numbers of maize hybrids, developed at Maize Research Institute-Kneja are tested in a wide range of environments (ECO trials). The screening period (2001-2015) covers 4 FAO groups (300-600), average number of hybrids for these trials are 18 for FAO 300-400, 41 for 400-500, 46 for 500-600 and 36 for full season types (600+). Number of locations vary from 3 to 6 by years, including irrigated and no irrigated ones. The highest grain yield was observed for the middle early group (FAO 400-500) – 7330 kg/ha^{-1}. Early types (FAO 300-400) and semi-late ones (FAO 500-600) have approximately the same productivity level – 7190 and 7165 kg/ha^{-1}, respectively. The late hybrids were less productive (FAO 600+) - 7057 kg/ha^{-1}.
The grain moisture at harvest increases for FAO groups – from 15.29% for early hybrids (300-400), 15.88% (400-500) 17.26% (500-600) and 18.95% for late ones (600+). Linear correlations were observed for performance index (p_i) and general adaptation index (x_i-b_i) values. They decrease from the early to the late FAO groups. The best combination of grain yield with fast dry down and stability was observed for maize hybrids from the early group (FAO 300-400). These results partially confirmed our previous investigations (Vulchinkov S. at al., 2013).
In spite of the influence of many accidental factors impact on these ECO trials – a different number of hybrids, locations and environmental conditions - a substantial tendency was found each year for all FAO groups. The grain yield increase of Bulgarian maize hybrids is 52.3 kg/ha^{-1} per year for the investigated 15 years period (fig. 1).
The rate of grain drying down is 0.32% per year (fig. 2). Both indicate that breeding progress was achieved.

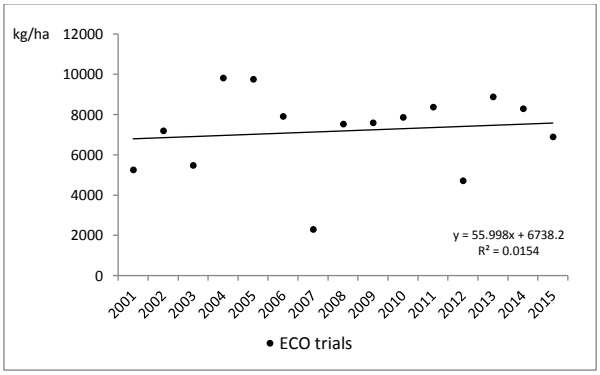

Fig.1. Grain yield tendency of maize hybrids for all FAO groups screening (2001-2015)

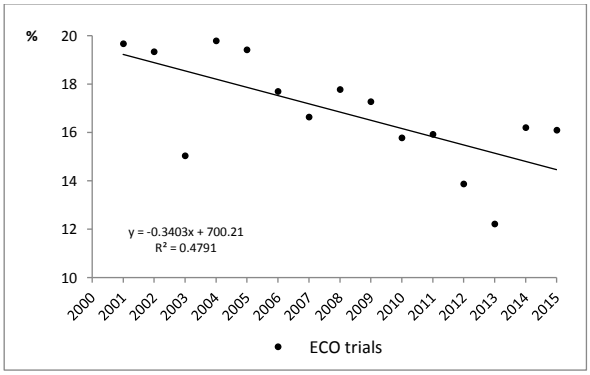

Fig.2. Grain moisture dynamics of maize hybrids for all FAO groups (2001-2015)

Genetic mapping of root traits in *Lolium perenne*

R. S. Yadav and **D. Allen**

Institute of Biological, Environmental and Rural Sciences (IBERS), Gogerddan, Aberystwyth, Ceredigion, United Kingdom

dka@aber.ac.uk

This work aims to identify markers/candidate genes linked with root morphological traits in perennial ryegrass. Identification of such markers/candidate genes will help develop future ryegrass varieties with efficient root systems. Improved root system is useful to plants not only in providing a superior anchorage but resistance to abiotic stresses such as to drought and flooding. To pursue such objectives, an F1 mapping population has been developed by crossing a genotype from the best performing IBERS perennial ryegrass variety (Abermagic) with a genotype from an old un-adapted variety (Aurora). A genetic linkage map of this population has been created with 3775 SNP markers. Highly significant differences in root number, thickness and in other root morphological traits were observed both in the parental genotypes of the mapping family as well as in the progeny of the mapping family. Analysis of root morphological data together with the SNP genotypic data in the progeny has identified a number of genomic regions associated with various root traits. Some regions of the genome harboured SNPs associated with with more than one root traits. Incidentally, traits such as root thickness, root numbers and root depth that showed significant phenotypic correlations of varying degrees had some QTLs in common. The basis of correlations observed will be discussed in the presentation. The presentation will also cover the candidate genes underlying the root morphological QTLs and their possible causal roles in determining the root phenotypes. Significance of the findings of the project in breeding improved root system in *Lolium perenne* will be discussed.

Parallel flash and poster presentations: Oil and protein (legume) crops

MicroRNA analysis of flax (*Linum usitatissimum* L.) genotypes in regard to alpha-linolenic acid content

Katarína Ražná[a] and **Lucia Hlavačková[a]**

[a] *Department of Genetics and Plant Breeding, Slovak University of Agriculture in Nitra, Slovak Republic*

lucia.hlavackova1@gmail.com

miRNAs (microRNAs), class of recently discovered non-coding endogenous RNA molecules, regulate gene expression post-transcriptionally. Due to their small size (21 – 24 nt), miRNA expression changes could be detected by qRT-PCR, which is accepted as a sensible, powerful technique in comparative expression analysis due to its high level of accuracy and practical ease. In terms of analyzing the genome plasticity, the flax genome has certain characteristics that make it an interesting research object. The aim of the study was to analyze polymorphism of selected miRNAs in flax linseed genotypes (Amon, Libra, Raciol) of different content of alpha-linolenic acid by functional markers based on miRNA molecules and by qRT-PCR. For the study two types of conserved miRNA families (miR156b and miR168) were selected. The family miR156b targets squamosa promotor-bindig protein, transcription factor in monocots and dicots and F-box protein sequences. One of the target sequences of miR168 family are sequences of cytochrome P450. The miR168 is considered as a biomarker of plant stress response. Polymorphism analysis based on miRNA molecular markers used the touchdown PCR method. A total of two miRNA-based forward primers and two universal miRNA reverse primers were combined together (miR156b-F/gm-miR-R, miR168-F/lus-miR-R and miR156b/miR168) to perform a marker assay. By the primers combinations miR156b/gm-miR-R and miR156b/miR168 it was possible to distinguish the genotype with the lowest content of alpha-linolenic acid (ALA) (Amon) from the genotypes with middle (Raciol) and high (Libra) content of ALA. miRNA expression was detected by qRT-PCR. On the basis of the average value of treshold cycle of miRNA and reference gene *UBE2* (Ubiquitin-conjugating enzymes E2), value $2^{-\delta CT}$ was calculated. Results based on this value suggest significant difference in miR156b activity of Amon genotype in comparison to genotypes Libra and Raciol. Within the miR168 family expression analysis was the difference recorded between genotype Libra and other two genotypes (Amon, Raciol). The most of miRNA families, including miR156 and miR168, are characterized by negative correlation between miRNA expression and expression of their target sequences. It means, that if the expression of a specific miRNA increased, the activity of target sequences regulated by this miRNA will be supressed and vice versa.

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This work has been supported by European Community under project no 26220220180: Building Research Centre "AgroBioTech" and by the project of Slovak Research and Development Agency APVV-0740-11 Understanding of Plant Adaptation in the Radioactive Chernobyl Area.

Antisense oligodeoxynucleotide treatment as a new method of gene expression manipulation in flax (*Linum usitatissimum*).

Anna Kostyn[a], Anna Kulma[b] and Jan Szopa[b]

[a] *Institute of Genetics and Microbiology, Faculty of Biological Sciences, University of Wroclaw, Przybyszewskiego 63/77, 51-148 Wroclaw, Poland*
[b] *Dept. of Genetic Biochemistry, Faculty of Biotechnology, University of Wroclaw, ul. Przybyszewskiego 63/77, 51-148 Wroclaw, Poland*

anna.kostyn@wp.pl

Terpenoids, also known as isoprenoids, are a group of natural products, which enclose primary and secondary metabolites. Terpenoids are the most varied class of plant natural products with carotenoids, tocopherols, gibberellins and sterols among them. They have a wide range of functions for plant growth and development. Terpenoids are strong antioxidants, thus they play an important role in photoprotection of photosystems and in regulation of plasma membrane fluidity. Moreover, terpenoids have antibacterial, antifungal and anti-cancer properties, therefore they are most desirable in human diet, and because of their antioxidative properties, they increase the stability of edible plant oils. Flax (*Linum usitatissimum*) is a crop valued for its fibre and seeds as a source of oil. Flax oil contains high quantities of polyunsaturated fatty acids, essential for human diet, though very prone to oxidation. Presence of antioxidants, such as terpenoids, in flax oil can decrease its susceptibility to the oxidative degradation. Although, flax is crop with active terpenoid pathway, desirable compound from this pathway are in small quantities in this plant. Therefore, study on the terpenoid pathway and its manipulation (by genetic engineering) is important for better understanding the metabolic flux and future application in food, medicine and pharmaceutical industries. The best method to investigate a gene role is transgenesis. Manipulations using agro- or biolistic transformations are time-consuming, require specialized equipment and are connected with GMO plant production. Therefore a new technique of gene expression modification employing oligodeoxynucleotides (AO, ASO), which are short, single stranded DNA molecules, complementary to target mRNA, was used. Application of ASO leads to changes of target gene expression in short time. This creates a possibility to manipulate expression levels of various genes from a pathway of interest. Antisense oligodeoxynucleotides were shown to be efficient in animal systems, however, their effect on gene expression in plants is poorly understood. We have designed ASOs for different genes encoding the enzymes of the terpenoid pathway (such as: isopentenyl diphosphate isomerase - IPPI, phytoene synthase - PSY, carotenoid isomerase - CRTISO, zeta-carotene desaturase - ZDS, tocopherol cyclase - VTE1) and used them for gene expression manipulation. It turned out that the antisense oligodeoxynucleotides method can be more effective than the traditional methods of transient gene expression modifications, such as biolistic transformation. Antisense oligodeoxynucleotide treatment allowed us to obtain the gene expression silencing even by 90% in comparison to the control plants. We demonstrate the effect of silencing with the new method on terpenoid pathway and its stability in flax.

Dissection of year related climatic variables and their effect on winter oilseed rape (*Brassica napus* L.) development and yield

Ana Marjanović-Jeromela[a], Sreten Terzić[a], Mirjana Jankulovska[b], Miroslav Zorić[a], Ankica Kondić-Špika[a], Milan Jocković[a] and Nikola Hristov[a]

[a] *Institute of Field and Vegetable Crops, Novi Sad, Serbia*
[b] *Faculty of Agricultural Sciences and Food, University of Skopje, Republic of Macedonia*

ana.jeromela@nsseme.com

Each phase of rapeseed development can be under the influence of yield-limiting processes that can hinder an improvement in yield, of which the climatic factors are one of the most frequent. The objectives of the present study were to evaluate the sources of variability for seed yield and oil content, to understand relations between the year related interactions and the effect of climatic variables in different growth stages, and to assess the presence and nature of cultivar by year (C × Y) and treatment by year (T × Y) interaction by multivariate analysis. Four rapeseed cultivars were evaluated (Jet Neuf, Banaćanka, Samurai and Falcon), during four growing seasons in two independent experiments with three sowing dates (SD) and five nitrogen rates (N). Six climatic factors were observed: minimum, minimum on 5 cm above ground, maximum and mean temperatures, total precipitation and relative air humidity, each calculated and averaged for the duration of individual growth stages: germination, overwintering, budding, flowering and ripening. A mixed effect split-split-plot analysis of variance was performed to estimate the significance of the main effects and respective interactions and factorial regression models to determine the effect of particular environmental variables on the year related interactions. TxY and CxY interactions in the N experiment were significant for both analyzed traits, while in SD experiment the interactions were significant only for oil content, indicating that these traits can be influenced by changing the N application rate or sowing dates or cultivars to achieve the best rapeseed performance. N rate caused significant differences in seed yield and oil content in rapeseed. The lowest yield was determined when no N fertilizer was applied (2.36 t ha^{-1}) and it increased by increasing the N rates. The increase in N rate from N_0 to N_{150} led to a decrease in oil content from 45.8 to 43.9%. The interaction patterns revealed by the multivariate analysis indicate that the analyzed cultivars in both experiments had different performance in different years and that there is no superior cultivar in all years of examination as most of the cultivars were grouped on the biplots according to the cultivation year. The application of different N dosages adversely affected seed yield and oil content, except in cultivar Banacanka which had the highest overall oil content (45.7%) and high seed yield (2.95 t·ha^{-1}), indicating that optimal dosage should be adapted to specific cultivars. In both experiments, relative air humidity at flowering and maximum temperature at the period of overwintering were identified as the most important factors for seed yield. Considering the TxY interaction, the largest proportion of explained interaction variance in the SD experiment was obtained for precipitation at overwintering (81.4 %) for oil content. In the N rate experiment, precipitation at budding stage (75.8%), relative air humidity at overwintering (63.3%) and flowering stage (53.0%) explained the highest proportion of TxY interaction for seed yield and precipitation at flowering (92.0%) and ripening (85.0%) for oil content. This study is the first to successfully dissect the influence of year related climatic variables on agronomical traits in winter oilseed rape. Based on the obtained information, appropriate agronomic practices can be applied at specific growing stages to ensure high seed and oil yield.

Marker assisted breeding of new winter oilseed rape lines (*Brassica napus* L.) with changed seed oil fatty acid composition

Katarzyna Mikolajczyk[a], Miroslawa Dabert[b], Stanislaw Spasibionek[a], Jan Bocianowski[c], Joanna Nowakowska[a], Teresa Cegielska-Taras and Iwona Bartkowiak-Broda[a]

[a] *Plant Breeding and Acclimatization Institute-NRI, Research Division in Poznan, Poland*
[b] *Faculty of Biology, Adam Mickiewicz University of Poznan, Poland*
[c] *Department of Mathematical and Statistical Methods, University of Life Sciences in Poznan, Poland*

kamik@nico.ihar.poznan.pl

Oilseed rape (*Brassica napus* L.) is a worldwide important oil crop, used for human nutrition as well as a source of raw material for biofuel production and for chemical and pharmaceutical industry. Oil crop market demands concern, among others, development of new cultivars characterized by the changed seed oil fatty acid composition comprising high oleic and low linolenic acid content, HOLL-type, applied for biofuel production due to its oxidation and thermal stability. At the Plant Breeding and Acclimatization Institute in Poznan, Poland, high oleic HO and low linolenic LL mutant lines were obtained by chemical mutagenesis (Spasibionek, 2006) and improved by recombinant breeding and introducing into new genetic background. While selection, routine biochemical analysis of seed oil fatty acid composition by gas-liquid chromatography was accompanied by the developed unique SNaPshot analysis using allele-specific functional genetic markers for precise monitoring of mutated and wild-type alleles of *FAD3* desaturase genes in the *B. napus* A and C genomes (Mikolajczyk et al., 2010; 2011; Bocianowski et al., 2012) involved in C18:3 linolenic acid synthesis. Furthermore, HO and LL oilseed rape lines were crossed to obtain HOLL recombinants, and application of specific genetic markers was necessary for effective selection, and especially in the case of low linolenic acid content (Spasibionek et al., 2015), as the phenotype expression of the trait is highly dependent on changing environmental conditions, *i.e.*, light and temperature. At present, marker assisted breeding, MAB, of HOLL genotypes of good agronomic value is in progress accompanied by screening of segregating populations with the use of allele-specific functional genetic markers.

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Identification of cinnamyl alcohol dehydrogenase isoforms in flax and preliminary assessment of their specificity in response to the biotic and abiotic stresses

Marta Preisner[a], Anna Kulma[a], Tadeusz Czuj[a] and Jan Szopa[a]

[a] *Department of Genetic Biochemistry, Faculty of Biotechnology, University of Wroclaw, Poland*

marta.preisner@gmail.com

Being a part of plant defence system and literally the mechanical barrier for pathogens, lignin is synthetized as a branch of the phenylpropanoid pathway. It also ensures mechanical properties of natural fibers. However, when it comes to application of plants and their products, lignin is a stumbling block. Thus, the research on the identification and characterization of genes responsible for lignification give ground for further research that will contribute to the diversification of plant applications, the emergence on the market new raw material as a source of biomass without the risk of negative effects of this manipulation, such as lowering the resistance of modified plants against pathogen infection. Flax (*Linum usitatissimum* L.) fiber is the strongest of the vegetable fibers, whose strength is two to three times greater that of cotton. However, fiber quality highly depends on retting time and the kind of microorganisms, the longer the exposition to atmosphere, the worse fiber's hue and texture and the more micro defects in the fiber's structure. In the past, because of a valuable fiber and oil, flax was widely grown in a temperate climate where cotton cannot be cultivated. The last enzyme that catalyzes synthesis of the lignin monomers is cinnamyl alcohol dehydrogenase (CAD). Structure and mechanism of action is well established for CAD, however, very recently it was reported that CAD family has a large number of members and can be divided into three main classes of which only one is responsible for vascular tissue development. It remains unclear, what are the roles of particular isoforms of CAD in lignification process, especially in response to the stress factors. The aim of the study was to identify the genes of all cinnamyl alcohol dehydrogenase (CAD) isoforms together with their promoters' sequences in fibrous flax (*Linum usitatissimum* L.). In the preliminary characterization, their specificity was determined by *in silico* analysis of their promoters to identify the *cis*-regulatory sequences that are induced/inhibited by the environmental factors. The obtained data were correlated with the level of expression of the isoforms in response to the biotic and abiotic stresses. It allowed determining the specificity of expression of the CAD gene isoforms and an indication of the molecular mechanism of the observed specificity.

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Evaluation of sunflower (*Helianthus annuus* L.) single cross hybrids under heat stress condition

Maria Khan, Maria Kausar and **Saeed Rauf**

Department of Plant Breeding & Genetics, University College of Agriculture, University of Sargodha, Pakistan

saeedbreeder@hotmail.com

Sunflower is an important oilseed crop. However, achene yield was often prone to heat stress. In present study, cytoplasmic male sterile lines selected for the differences between post and pre noon canopy temperature (Δ) during early segregating generation were crossed with restorers to generate 64 single cross hybrids which were then evaluated under heat stress condition for two years and compared with the standard hybrids. Results showed that single cross hybrids had substantial genetic variability for yield under heat stress condition. Moreover seed yield per head could be directly used to differentiate single cross hybrids. Genotype environment and genotype was used to differentiate single cross hybrids on the basis of multiple traits. GGE biplot showed that several single cross hybrids had higher seed yield potential than standard check. Moreover, seed yield per head was closely related with pollen viability showing that yield under heat stress was product of high gametophytic fertility. Hybrids having high seed yield potential under heat stress had lower cell membrane injury (CMI) showing that potential hybrids could be selected on the basis of CMI during seedling stages. GGE biplot for seed yield head^{-1} and its components showed that single could be characterized into two major groups on the basis of yield components. Group Ia included hybrids with high 100-SW, while group Ib had the hybrids with high number of achenes head^{-1} and head diameter. Group II had the hybrids with high kernel weight and kernel to achene ratio. The hybrids could be recommended according to their potential utilization in the seed industry.

Global inventory and evaluation of wild perennial cereal, pulse and oilseed species for pre-breeding and domestication

Claudia Ciotir[a,b], Wendy Applequist[b], Tim Crews[c], Lee DeHaan[c], Sterling Herron[a,b] , Andrew Townesmith[b], David Van Tassel[c], and **Allison J. Miller**[a,b],

[a] *Saint Louis University/Department of Biology, USA*
[b] *Missouri Botanical Garden Department of Research and Conservation,USA*
[c] *The Land Institute, USA*

amille75@slu.edu

The Perennial Global Inventory Project (PGIP) is a collaborative project between Saint Louis University, the Missouri Botanical Garden (St. Louis Missouri), and The Land Institute (Salina, Kansas). Perennial species offer deeper rooting depths, reduced erosion risks, greater interception, retention, and utilization of precipitation, and improved ecosystem functioning relative to annuals (Jackson 1980; Glover et al. 2010). PGIP aims to systematically identify and evaluate perennial wild herbaceous and subshrubby species in the Asteraceae, Fabaceae, and Poaceae for inclusion in pre-breeding programs including wide hybridization with existing crops and de novo domestication (DeHaan et al. 2014). The long-term goal of this project is to expand the use of perennial members of these families in agriculture, in order to develop perennial grain crops. Although perennial Asteraceae, Fabaceae, and Poaceae occur in nature, these species were rarely domesticated (Van Tassel et al. 2010).

Plant biodiversity information housed in botanical gardens represents an important resource for the identification of promising species that promote food and ecosystem security (Miller et al. 2015). For centuries, gardens have collected detailed information on plant identification, geographic distributions, morphology, phenology, reproduction, and traditional uses. Botanical gardens can serve as a valuable bridge connecting information about naturally occurring plant diversity with the agricultural research community. Gardens can also serve as a source of seed for experimental populations thereby reducing the impact and cost of seed collection from wild populations.

PGIP aims to develop an online database of perennial, herbaceous Asteraceae, Fabaceae, and Poaceae integrating information on taxonomy, geographic distribution, reproductive biology (especially sexual reproduction traits), and ethnobotany, among others. These data are being collected from various sources including floras, wild germplasm inventories, conservation catalogs, and scientific publications. The database will exist as a special project within TROPICOS, the taxonomic and specimen database developed by the Missouri Botanical Garden. The PGIP leverages extensive scientific expertise at The Land Institute and the Missouri Botanical Garden to identify promising perennial herbaceous grass, legume, and oilseed species that have potential for use as human edible seed crops. PGIP is designed as a multifunctional, efficient, and productive tool that will stimulate interest in perennial crops and will promote interactive partnerships with the scientific community of EUCARPIA. The online database resulting from this project will link to other crop wild relative and germplasm databases, and will be freely available to the public but designed for a broad user community including plant breeders, seed bank scientists, taxonomists, conservationists, policy-makers, farmer networks, and the wider public.

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Physiological responses of soybean cultivars under iron deficiency: a case study in Turkey

Emre Aksoy[a], Sevgi Çalışkan[b] and **İlknur Tındaş[a]**

[a] *Department of Agricultural Genetic Engineering, Nigde University, Turkey*
[b] *Department of Plant Production and Technologies, Nigde University, Turkey*

ilknur4888@hotmail.com

Iron (Fe) is one of the essential micronutrients for both plants and humans, and Fe deficiency is among the most widespread nutritional deficiencies. Fe deficiency leads to Fe deficiency chlorosis (IDC) due to decreased chlorophyll biosynthesis, which, in turn, directly causes yield losses in plants. Soybean (*Glycine max* L.) belongs to the legume family and is the top second plant species with the highest Fe content. However, soybean yields are negatively affected by Fe deficiency during growth in the field. The loss in soybean yields due to IDC is predicted to be millions of tons every year only in the USA. Although IDC in soybeans has been observed for years, the studies to develop IDC-tolerant soybean cultivars were slower compared to the studies of other plant species, and many of these soybean studies solely depend on physiological observations. Recommended soybean varieties have been grown in different parts of Turkey suitable for soybean farming depending on their maturation groups; however, farmers trying soybean for the first time dropped out its farming due to yield losses resulted from variety maladaptation. One of the reasons for soybean farmers to leave its farming may be predicted as they did not use soybean varieties with high yields and tolerance to Fe deficiency, and are suitable for farming in soils of Turkey since majority of soils in Turkey are potentially Fe deficient. Therefore, it is highly necessary to analyse the accumulation levels of Fe in leaves and seeds of soybean varieties grown in Turkey, and make a correlation analysis between these levels and IDC status. Unfortunately, physiological and biochemical responses of various soybean varieties grown in Turkey under Fe deficiency have not been studied yet. In a preliminary field trial experiment in Nigde, Turkey, where calcareous soil is abundant, some soybean varieties were observed to be greener than the others. This observation was also confirmed in SDAP analyses. It is known that SPAD measurements correlate with the clay amount in the soil, suggesting the genotypes with high SPAD values may accumulate more iron, therefore tolerate iron deficiency better. In order to increase the soybean yields grown in Turkey, IDC tolerance and Fe levels in leaves and seeds of 20 soybean varieties (in different maturation groups) will be further investigated.

Flowering time diversity for U.S. groundnut mini-core collection produced under Mediterranean climate type

Engin Yol and Bulent Uzun

Department of Field Crops, Faculty of Agriculture, Akdeniz University, Antalya, Turkey

enginyol@akdeniz.edu.tr

The selection of early maturity cultivars is one of the important objectives in most of the groundnut breeding programs. It enables escape from drought conditions and to fit in multiple cropping systems. Flowering time is a significant component of early maturity and potentially useful in designing an effective breeding strategy for developing early-maturing cultivars (Upadhyaya and Nigam 1994). This trait shows wide variability and therefore each genotype should be re-evaluated with respect to time of flowering in different regions. In this perspective, the U.S. groundnut mini-core collection were investigated with regard to flowering time in Antalya, Turkey, with a true Mediterranean-type environment. The traits of days to first flowering and days to 50% flowering were used to characterize flowering time. Results showed that days to first flowering ranged from 23 to 48 days in the collection. The earliest flowering was observed in PI 493938, PI 482120, PI 481795, PI 493717 and PI 493938; the latest flowering was observed in PI 496448. Grand mean for the character first flowering was 31 days. The other flowering trait, days to 50% flowering, showed a great variation from 26 (PI 493938) to 55 (PI 496448) days. The genotypes showed early flowering in the collection would be selected to improve earliness for shorter season production in Mediterranean type environments. Beside, earliness may allow groundnut to post-wheat second crop cultivation at similar climatic areas. Agro-morphological assessment would be the next step to develop high yielding and early flowering groundnut cultivars suitable for this kind of regions.

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Molecular cytogenetic analysis of three generations of resynthesized rapeseed (*Brassica napus* L.)

Alexandra V. Amosova[a], Lyudmila V. Zemtsova[a], Elena N. Zhidkova[b], Anatolii A. Muravlev[b], Olga Yu. Yurkevich[a], Tatiana E. Samatadze[a], Tomasz Książczyk[c], Valentina T. Volovik[d], **Anna V. Shirokova**[e], Svyatoslav A. Zoshchuk[a] and Olga V. Muravenko[a]

[a]*Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Russia*
[b]*All-Russian Rapeseed Research Institute, Federal Agency for Scientific Organizations, Russia*
[c]*Institute of Plant Genetics, Polish Academy of Sciences, Poland*
[d]*All-Russian Williams Fodder Research Institute, Russia*
[e]*Koltzov Institute of Developmental Biology, Russian Academy of Sciences, Russia*

glandularia@yahoo.com

Interspecific hybridization is one of the breeding methods which are used to broaden the genetic diversity of polyploid plants and produce valuable crops with desired properties. *Brassica napus* L. (AACC; 2n = 38) is a natural allopolyploid formed by spontaneous interspecific hybridization between *B. oleracea* L. (CC; 2n=18) and *B. rapa* L. (AA; 2n=20) followed by diploidization. It is also possible to produce resynthesized rapeseed lines (usually with unstable genomes) via an artificial cross between the species of the genus *Brassica* containing progenitor diploid genomes (A and C) of *B. napus*. Natural and resynthesized *B. napus* are fine models for studying reorganizations of parental genomes occurring in hybrid genomes of succeeding generations. In the present study, the molecular cytogenetic analysis of F_3, F_7 and F_8 generations of a resynthesized rapeseed line (*B. rapa* ssp. *narinosa* x *B. oleracea* ssp. *capitata*) produced by selection of early-growing and yellow-seeded *B. rapa*-type progeny was performed by FISH with 45S and 5S rDNA, C-subgenome specific probe (BoB014O06 BAC clone from *B. oleracea* BAC library) and GISH with labelled total DNA of *B. rapa*. This approach allowed us to identify all the individual small-sized chromosomes in rapeseed karyotypes and also to determine their subgenomic affiliation. According to our observations, the chromosome number in karyotypes of hybrids reduced from 38 in F_3 to 20-24 (20 was a modal number) in F_7 and F_8 that corresponded to the number of chromosomes in A-genome (*B. rapa*). In F_3, FISH analysis revealed common rapeseed karyotypes including 12 chromosomes with 45S rDNA sites and 12-14 chromosomes with 5S rDNA. In karyotypes of F_7 and F_8, pericentromeric location of co-localized 45S and 5S rDNA, the presence of a small chromosome with a telomere 5S rDNA site (peculiar to A-subgenome) and absence of a large chromosome with 1-2 5S rDNA sites in the long arm (peculiar to C-subgenome and found in F_3 karyotypes) showed that most of the chromosomes belonged to parental A-genome. FISH with BoB014O06 clone did not reveal any hybridization sites in 20-chromosomal karyotypes in F_7 or F_8 though, in karyotypes with 21-24 chromosomes, the sites of hybridization were detected on 1-4 chromosomes. GISH analysis with labelled DNA of *B. rapa* detected all the 20 chromosomes in karyotypes of F_3, F_7 and F*8* hybrids confirming their affiliation to A-genome. Thus, selection of early-growing and yellow-seeded *B. rapa*-type progeny leads to gradual deletion of C-genome chromosomes (*B. oleracea*), and, mostly, chromosomes of A-genome maintained in karyotypes of F_7 and F_8. The findings demonstrate that progeny selection of valuable traits in unstable resynthesized rapeseed might lead to deletion of parental genome chromosomes, and karyotype examination is necessary. The approach can be useful for obtaining chromosome addition lines which are important for genetics and selection.

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Evaluation of selected cowpea lines for agronomic performance and use in breeding program in South Africa

Joseph A.N. Asiwe[a], Osman Boukar[b] and Remi A. Adeleke[b]

[a] *Department of Plant Production, Soil Science and Agricultural Engineering, University of Limpopo, Sovenga, South Africa*
[b] *Cowpea breeding, International Institute of Tropical Agriculture, Ibadan, Nigeria*

joseph.asiwe@ul.ac.za

Cowpea (*V. unguiculata L. Walp*) is an important source of protein rich food and fodder in many countries of the tropics and sub-tropics. However, due to the lack of improved early maturity varieties, cowpea cultivation in South Africa is very limited especially in the drought-prone areas of Limpopo province. Therefore, concerted efforts are being made to select cowpea varieties that combine early maturity, high yield and pest resistance. In 2015, a total of 21 cowpea breeding lines and varieties from the International Institute of Tropical Agriculture (IITA) and five varieties including local check Glenda, from South Africa, were evaluated at the University of Limpopo Research farm, Syferkuil. Data collected include number of days to maturity, grain and fodder yield and hundred seed weight. Results showed significant ($P < 0.05$) differences for all the variables measured and majority of the breeding lines performed better than Glenda. Number of days to plant maturity varied from 78 - 95 days and 13 varieties matured earlier than Glenda (83 days). Grain yield varied from 329 - 2501 kg · ha^{-1}. IT11D-61-82, IT10K-836-2, IT10K-836-4, lines 6-3-1, 6-2 and 6-2-1 and IT00K1263 were top seven performers and 19 test lines out-performed the local check, Glenda. The fodder yield ranged between 400 and 4266.7 kg ha^{-1} and Jana Fod produced the highest fodder yield. Hundred seed weight ranged between 11 and 26 g, Varieties IT11D-61-82, IT10K-836-2, IT10K-836-4 and IT10K-837-1 and lines 6-2 and 6-2-1 had the largest seed size (20-26 g). Based on yield and maturity as well as seed type, six varieties (lines 6-2 and 6-2-1, IT11D-61-82, IT10K-836-2, IT00K1263 and Jana Fod) were selected for further multi-location testing and for use in cowpea breeding program.

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Variability of sterols content in oil of white mustard (*Sinapis alba* L.)

Iwona Bartkowiak-Broda[a], Magdalena Rudzinska[b], Elżbieta Radziejewska-Kubzdela[b], Teresa Pietka[a] and Krzysztof Michalski[a]

[a] *Plant Breeding and Acclimatization Institute – National Research Institute, Department of Oilseed Crops, Poland*
[b] *Faculty of Food Science and Nutrition, University of Life Sciences, Poland*

ibart@ihar.nico.poznan.pl

Researches on white mustard quality traits have been conducted in Plant Breeding and Acclimatization Institute for 30 years. The first achievement was variety without erucic acid in oil, Bamberka, licenced in 2006. The next step was the development of double improved variety Warta (licenced in 2011), characterized by very low erucic acid content in oil (below 1.5%), lack of sinalbin – the main glucosinolate in seeds of white mustard and very low content of other glucosinolates (below 15 μM/g of seeds). Nowadays, the aim of investigations is the development of double low variety with very high content of antioxidants in oil. The qualitative and quantitative composition of sterols: brassicasterol, campesterol, sitosterol, sitostanol, δ5-avenasterol, δ7-avenasterol was studied in seed oil of 100 double low white mustard lines using gas chromatography. With the use of HPLC was evaluated content of carotenoid – lutein. The highest variability was found in the case of campesterol and sitosterol. The investigated lines displayed very high variability in the total content of sterols in the range of 2473-17462 μg/g in oil. In the case of lutein, the variability for the material investigated was very low. The obtained results indicated the possibility of development of white mustard with very high content of sterols in oil which will be characterized by high oxidative stability and very valuable for human dietary needs.

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Response of glucosinolate (GSL) accumulation to sulphur in low and high GSL lines of *Brassica juncea*

Priyakshee Borpatra Gohain[a], Terry J. Rose[a] and Graham J. King[a]

[a] *Southern Cross Plant Science, Southern Cross University, Lismore, NSW, Australia*

p.borpatra.gohain.10@student.scu.edu.au

Brassicas are characterised by the co-production of sulphur (S) rich nitrogen containing glucosinolates (GSLs) and the enzyme myrosinase. When combined through tissue damage myrosinase acts on GSLs to release isothiocyanates, nitriles and epithionitriles, which have a range of distinctive mustard flavours, insect anti-feedant or attraction properties, as well as tumour suppression property in mammals. There are many lines of evidence suggesting that the plant S status significantly effect on its GSL content. Few studies have been carried out to elucidate the mechanism(s) behind the differential response of plant GSL content to varying S status. We are particularly interested to understand how low- and high-GSL lines respond to different doses of S fertiliser. In this study we have applied different rates of S nutrition to high- and low-GSL inbred lines of *Brassica juncea* to test the hypotheses that (i) increase in seed GSL content occurs at S fertiliser rates beyond that required for maximum seed yield and (ii) response of seed GSL to S availability varies with genotypes.

The variation of bioactive compounds in black and yellow seeds of winter oilseed rape (*Brassica napus* L.)

Teresa Cegielska-Taras[a], Małgorzata Nogala-Kałucka[b], Laurencja Szała[a], Aleksander Siger[b]

[a] *Plant Breeding and Acclimatization Institute - Research Division Poznań, Department of Genetics and Breeding of Oilseed Crops, Poznan, Poland*
[b] *University of Life Sciences, Faculty of Food Science and Nutrition, Department of Biochemistry and Food Analysis, Poznan, Poland*

tceg@nico.ihar.poznan.pl

In the study an analysis of tocopherols, plastochomanol-8 and phytosterols, was conducted using black-, brown- and yellow-seeded doubled haploid (DH) lines of winter oilseed rape (*Brassica napus* L.). DH line populations marked: ZH and HZ, were obtained from F_1 hybrids of reciprocal crosses between two DH lines: DH Z114 yellow- and DH H_2 26 black-seeded. The fat content of the black-seeded parental line was 49% and this was higher than that of the yellow-seeded parental line (44%). The fat content of two DH line populations ranged from 44 to 51%. Total tocopherol content for the two studied populations ranged from ca. 460 to 602 mg kg^{-1} and the α-T/γ-T ratio was from 0.66 to 1.09. However, in parental lines DH $H_2$26 and DH Z114 total tocopherol content was 534 mg kg^{-1}, and 525 mg kg^{-1}, but the α-T/γ-T ratios were 0.81 and 1.21, respectively. The yellow-seeded parent line was characterised by higher contents of PC-8 (81 mg kg^{-1}) than black-seeded parental lines (58 mg kg^{-1}). The largest part of the total phytosterol content in seeds of both populations was β-sitosterol, i.e. from 976 mg kg^{-1} to 2148 mg kg^{-1}, followed by campasterol, from 636 mg kg^{-1} to 1364 mg kg^{-1}, and brassicasterol from 375 mg kg^{-1} to 678 mg kg^{-1}. Significantly positive correlations were observed between the seed colour with α-T as well as α -T/γ-T ratio and PC-8 content. In contrast, the correlations between the seed colour with total tocopherol and total phytosterol content were not noted.

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Screening of soybean germplasm for *Rsv4*, a gene conferring Soybean mosaic virus resistance, using SSR markers

Yu Mi Choi[a], Do-Yoon Hyun[a], Sukyeung Lee[a], Myung-Chul Lee[a], Sejong Oh[a], Ho-Cheol Ko[a] and On-Sook Hur[a]

[a] *National Institute of Agricultural Sciences, Rep. of Korea*

cym0421@korea.kr

Soybean mosaic virus(SMV) is one of the devastating frequent diseases in soybean, causing yield loss and seed deterioration. The best method to control viral disease is the use of resistant cultivars, which can be obtained from genetic resources, possessing SMV resistance. Marker-assisted selection has been generally used for disease resistance. Markers such as simple sequence repeat (SSR) have been developed on the basis of whole-genome sequence of soybeans. Some of them are closely related to resistance genes conferred to SMV resistance.
Seven strain groups (G1 - G7) and three independent loci, *Rsv1, Rsv3* and *Rsv4,* are known for SMV resistance in soybean. *Rsv1* confers resistance to some (G1 - G6), *Rsv3* conditions resistance to SMV G5 through G7, *Rsv4* provides resistance all 7 SMV strains at the seedling stage. *Rsv4* is an important gene because of its resistance to all 7 strain groups of SMV and its mode of action to inhibit the hypersensitive-response caused by *Rsv1* alleles. The objective of this study was to select SSR markers to screen soybean germplasm with *Rsv4,* a gene conferring SMV resistance, to evaluate the soybean germplasm using the selected markers and to choose useful resources for *Rsv4* genes.
The two differential soybean genotypes used in this study were PI 486355 carrying *Rsv4* and Lee68 carrying *rsv4*. Ten SSR markers (AI856415, Satt095, Satt157, Sat_254, Satt266, Satt296, Satt542, Satt558, Satt634 and Satt698) were used for choosing soybean germplasm containing *Rsv4*. Four of them were selected on the basis of polymorphism, which were Sat_254, Satt266, Satt634 and Satt698. We could distinguish susceptible from resistant plants using these markers, which were applied to 348 soybean accessions from Korea and abroad. We could select 83 accessions using Sat_254, 97 accessions using Satt698, 195 accessions using Satt266 and 48 accessions using Satt634 for containing *Rsv4* alleles. To reconfirm, we selected germplasm to show the corresponding results for 4 markers. As a result, 10 accessions (IT144019, IT157861, IT022041, IT022168, IT170841, IT170961, IT283925, IT274316, IT274882 and IT276019) were selected as a germplasm carrying *Rsv4* gene. Further, we are going to do biological tests to confirm that these genotypic results are identical with the phenotypic, and through these results we will establish the mass screening system for soybean germplasm with *Rsv4* gene. Our final goal is to use soybean germplasm to develop new cultivars with SMV resistance

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Legumes germplasm collection at the MBG-CSIC, Spain

Antonio M. De Ron[a] and María del Carmen Menéndez Sevillano[b]

[a] *Misión Biológica de Galicia (MBG)-Biology of Agrosystems, National Spanish Research Council (CSIC), Pontevedra, Spain*
[b] *National Agricultural Technology Institute (INTA)-Active Bank of Northwestern Argentina (BANOA), EEA Salta, Argentina*

amderon@mbg.csic.es

The common bean (*Phaseolus vulgaris* L.) is the most important grain legume for direct human consumption on a global scale. This crop has spread to every continent over the past centuries, which resulted in a complex genetic structure of bean germplasm outside its areas of origin and domestication (South and Central America) (Santalla et al. 2002). The current Spanish landraces are the result of selective pressure and phenotypic selection by farmers and they are currently well adapted to the agroecological conditions under they have been grown for centuries. For these reasons the main legume crop maintained at the MBG-CSIC collection is the common bean. The common bean collection started in 1987 (De Ron et al. 1991) and comprises 2014 accessions from different countries: Europe (17 countries), The Americas (15) (Menéndez Sevillano, Asia (4), Africa (1) and Oceania (1). The genetic stock includes about 500 breeding lines and RILs (Recombinant Inbred Lines). A core collection was built in 2003 including 52 Spanish accessions (Rodiño et al. 2003). The runner bean (*Phaseolus coccineus*) collection started also in 1987 and currently has 49 accessions.
Cowpea (*Vigna unguiculata*) collection started in 1987, together with the collections of *Phaseolus*, since in many places these crops grow together in the farmers' fields. The collection includes 91 accessions: 43 from Spain and 48 from Portugal. The pea (*Pisum sativum*) collection also started in 1987. The main use of pea in Spain is as vegetable, both for the fresh grain and for the immature pod. Recently the interest of this crop is being based in its use for animal feed as source of protein. The current collection includes 164 accessions, being 123 from Spain. Lupin (*Lupinus* spp.) collection started in 1999. Mostly of the accessions are wild, collected in field. Currently the collection includes 210 accessions, mainly from Spain: *L. angustifolius* - 90, *L. hispanicus* - 43, *L. luteus* - 73 and *L. albus* - 4. The chickpea (*Cicer arietinum*) small collection, started in 2015, includes only 14 accessions from Spain.

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Characterizing genes isolated from tetralocular ovary of *Brassica rapa* by RNA-seq

Jung Sun Kim, Mi-Sook Seo, So Youn Won, Sang-Ho Kang, Seong-Han Sohn

Department of Agricultural Biotechnology, National Institute of Agricultural Sciences, RDA, JeonJu, 54874, Republic of Korea

jsnkim@korea.kr

The genus *Brassica* contains some of the most important vegetable and oleiferous crops of the world. *Brassica* flowers usually have bilocular ovary with an average 20~24 seeds per silique after fertilization. Rarely some *B. juncea* and yellow sarson (*Brassica rapa* ssp. *tricolari*s) plants have multilocular ovary with three or four locules. Plants with tetralocular ovary usually show increasd seed yields, making this an interesting trait for *B. rapa* breeding. Examining cross sections of tetralocular ovary under the microscope, we could show that the floral meristem of one particular yellow sarson, tetralocula ovary plant (LP8) already has four locules within 1mm immature buds. To identify genetic elements determining tetralocular ovary formation, RNA-seq was carried out from the isolated RNA from flower buds smaller than 1mm and larger than 2mm, respectively. For comparison, RNA from flower buds of the *B. rapa* cultivar Chiifu, which only produces bilocular ovary, was used. A total of 994 differentially expressed genes (DEGs) are detected in only LP8. Among the DEGs, we identified 18 DEGs in only immature buds smaller than 1mm. The expression patterns of 18 DEGs were validated by real time quantitative PCR. We finally cloned and sequenced 17 tetralocula ovary formation candidate genes from LP8. In comparison to sequences from bilocular Chiifu, we observed a large number of SNP and larger insertions / deletions. Now we will use binary vector constructs containing these DEG genes for genetic transformation for *A. thaliana*, *B. rapa*, and *B. napus* respectively. Our results will be helpful for understanding the mechanisms of tetraovular ovary formation in *Brassica rapa*.

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Identification and characterization of NF-YA7 gene in potato

Dool-Yi Kim[a], Kyung Hwa Kim[a], Soo-Kwon Park[a] and Jung-Kyung Moon[a]

[a] *Crop Foundation Division, National Institute of Crop Science, RDA, Jeonbuk, Korea*

dykim22@korea.kr

Potato (*Solanum tubersosum* L.) is susceptible to various environmental stresses such as frost, high temperature, and drought. In particular, potato tuber growth is greatly affected by drought, which causes not only yield reduction but also loss of tuber quality. Since unpredictable global weather changes cause more severe and frequent water limiting conditions, improvement of potato drought tolerance can minimize such adverse effects under drought and can impact on sustainable potato production. Genetic engineering can be utilized to improve potato drought tolerance, but such approaches using endogenous potato genes have rarely been applied. Here, we screened twelve potato genes from microarray analysis under drought and salt stress, and their expression patterns were re-examined at various stress conditions, involving exposure to PEG, NaCl, ABA, and low temperature. Nine and five genes were upregulated by PEG and NaCl, respectively. Among them three genes, *StNF-YA7* and two *LEA proteins* (*EM1* and *LEA2*) were fused to *smGFP* and expressed transiently in tobacco leaves. EM1- and LEA2-smGFP proteins were detected in nucleus and plasma membrane similar to free smGFP, whereas StNF-YA7-smGFP localized specifically in nucleus. *35S:StNF-YA7-smGFP* transgenic lines showed enhanced drought tolerance, and water loss in detached leaves of *35S:NF-YA7-smGFP* transgenic plants was less than that of wild type, suggesting that enhanced drought tolerance of *35S: StNF-YA7-smGFP* transgenic plants is likely due to reduced water loss through stomatal pores. *StNF-YA7* promoter induced vascular-tissue specific GUS expression.

Classification on maturity group and maturity locus by genotyping of Korean soybean cultivars

Kil-Hyun Kim[a], Min-Jung Seo[a], Myoung-Ryoul Park[a], Sang-Gon Kim[a], Hwann-Hee Bae[a], Jin-Seok Lee[a], Jung-Tae Kim[a], Beom-Young Son[a], Seong-Bum Baek[a], Jung-Kyung Moon[b], Jeom-Ho Lee [a] and Chang-Hwan Park[a]

[a] *National Institute of Crop Science, RDA, Suwon, Republic of Korea*
[b] *National Institute of Crop Science, RDA, Wanju, Republic of Korea*

pelicks@korea.kr

Soybean (*Glycine max* (L.) Merill) is a short-day crop with high protein and oil contents. Flowering time of soybean is controlled by interactions among photoperiod, temperature, and genotype. Short-day and high temperature promote flowering, whereas long-day and low temperature delay flowering. To date, nine maturity loci (*E1-E8* and *J*) have been reported to control flowering and maturity in soybean. Among them, four loci (*E1-E4*) are characterized by map- or candidate-based cloning. However, genotypes of Korean soybean cultivars have not identified for these loci. In this study, we performed phenotyping and genotyping of 61 Korean elite soybean cultivars classified by the usages which were soybean sprout, cooking with rice, soy-paste, and vegetable soybean. Maturity-related traits were also investigated. These soybeans were separated into three main groups which were early (35 days), mid (48 days), and late (60 days) on the basis of the flowering time. Four loci of 61 Korean soybean cultivars were genotyped by using 15 allele-specific DNA markers described by Zhai *et al* (2014). These cultivars had diverse flowering and maturity dates and showed various allelic combinations to adapt different latitudes. Most of soybeans for sprout, cooking with rice, and soy-paste were mid- or late-flowering cultivars, whereas vegetable soybeans were early-flowering cultivars. Interestingly, there were no soybean cultivars with recessive *e4* alleles suggested by the importance for high latitude adaptation (Jiang *et al*., 2014). Although phenotypic data did not exactly match with genotypic data, the recessive allele *e1* only existed in early-flowering cultivars. This result is consistent with previous studies that showed *E1* as the crucial component for elucidation of soybean flowering time regulation (Xia *et al*., 2012). Overall, this study could provide helpful information about identification of new genes involved in flowering time and maturity as well as marker assisted selection in soybean breeding programs.

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Fusarium oxysporum infection in flax results in salicylic acid level increase associated with phenylpropanoid pathway activation

Kamil M. Kostyn[a], Aleksandra Boba[a], Anna Kulma[a] and Jan Szopa[a]

[a] *Dept. of Genetic Biochemistry, Faculty of Biotechnology, University of Wroclaw, Wroclaw, Poland*

kostyn@ibmb.uni.wroc.pl, kamilkostyn@o2.pl

Flax (*Linum usitatissimum* L.) is a crop plant valued for its oil and fibre. Unfortunately, large losses in cultivation of this plant are caused by fungal infections, with *Fusarium oxysporum* being one of its most dangerous pathogens. Among the plant's defence strategies, changes in the expression of genes of the shikimate/phenylpropanoid/benzoate pathway and thus in phenolic contents occur. Among the benzoates, salicylic acid and its methylated form methyl salicylate play an important role in regulating plants' response to stress conditions. We investigated the expression of selected genes of the phenolic biosynthesis pathway in the first stages after *F. oxysporum* infection. Upon treatment of flax plants with the fungus, we found that salicylic acid content increased (100% above the control). The expression profiles of the analysed genes suggested that it is produced most likely from cinnamic acid, through the β-oxidative route rather than through isochorismate synthase controlled pathway. Phenylalanine lyase gene transcript level increase reached 8-fold of the control, while 4-coumarate-CoA ligase expression reached over 2-fold increase at 12 hours post infection. Beta-ketothiolase transcript level was 1.5-fold of the control and this increase persisted throughout all timepoints investigated (from 6 to 48 hours post infection). At the same time no changes of isochorismate synthase gene transcript level were observed. Moreover, increased expression levels of the genes involved in lignin biosynthesis were observed, which correlated positively with higher lignin content after infection (by 21%). We suggest that the gene expression is controlled by salicylic acid as part of the plant's response to pathogen attack.

Genome-wide association study for common bean phenolic compounds in Portuguese germplasm

Susana T. Leitão[a], Elsa Mecha[a], Mara L. Alves[a], Geoffrey Istas[b], Rodrigo Feliciano[b], Ana Rodriguez-Mateos[b], Maria do Rosário Bronze[a,c,d] and Maria Carlota Vaz Patto[a]

[a] *ITQB-NOVA, Oeiras, Portugal*
[b] *Division of Cardiology, Pulmonology, and Vascular Medicine, Medical Faculty, University of Düsseldorf, Germany*
[c] *iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal*
[d] *iMED, Faculdade de Farmácia, Universidade de Lisboa, Portugal*

sleitao@itqb.unl.pt

Common bean (*Phaseolus vulgaris* L.) is the worldwide most important grain legume in human diet. The consumption of common bean has recognized benefits, not only in a nutritional perspective, but also due to its nutraceutical properties. Potential health benefits of common bean have been mainly attributed to the presence of secondary metabolites such as phenolic compounds with antioxidant activity.

Portugal holds a very promising common bean germplasm resulting from more than five centuries of adaptation to local environmental conditions and to quality mass selection by farmers. This germplasm may represent important sources of interesting traits, such as nutritional quality for food, not yet fully explored in breeding.

In order to pave the way for common bean nutritional quality improvement based on this valuable, highly diverse and unexplored genetic resource, we have searched for the genomic regions controlling these phenolic compounds concentration.

Common bean extracts of a collection of 170 Portuguese accessions were analyzed using Q-TOF-LC-MS in order to identify and quantify phenolic compounds. As a result, 9 phenolic compounds were identified, namely *t*-ferulic acid, *p*-coumaric acid, caffeic acid, 4-hydroxybenzoic acid, protocatechuic acid, gallic acid, kaempferol, *(+)*-catechin and epicatechin.

The same collection was screened using 62 SSRs, an Illumina BeadChip of 5398 SNPs and a DArTseq array with 12k SNPs, uniformly distributed throughout the genome. Population structure and its genetic diversity were investigated.

To detect significant associations between the common bean phenolic compounds found and the molecular markers, a genome-wide association study, joining the phenotypic information from the phenolic profile of two years of field experiments with the genotypic information, is currently ongoing. A mixed linear model analysis, including population structure and familial relatedness, is being used to identify significant associations. Results will be reported and discussed.

This work will contribute to the development of common bean varieties with better nutritional quality, meeting consumers' and farmers' expectations at the same time that support the improvement and production of an underused resource with a vital role in sustainable agriculture.

Evaluation of genetic diversity of broad bean genetic resources in the Baltic Region

Līga Lepse[a], Arta Kronberga[b], Margit Olle[c], Solvita Zeipiņa[a], Aina Kokare[b] and Ināra Konošonoka[b]

[a] *Pūre Horticultural Research Centre, Latvia*
[b] *Institute of Agricultural Resources and Economics, Latvia*
[c] *Estonian Crop Research Institute, Estonia*

liga.lepse@puresdis.lv

A traditional crop in the Baltic region, faba bean (*Vicia faba* L.) is becoming increasingly popular due to its nutritional value as a protein source for food and feed and for the agricultural services it provides – biological nitrogen fixation, promotion of soil biological activity and use as a break crop in monoculture systems. In the Baltic region, broad beans are used as a grain legume in agriculture (mostly *Vicia faba* var. *minor*) and also as a vegetable crop in gardens (mostly *Vicia faba* var. *major*).

During the first two years of the implementation of the FP7 project EUROLEGUME (2014 and 2015), local genotypes and landraces of faba bean were investigated in terms of their genetic diversity and productivity parameters. Samples of locally grown commercial varieties and old cultivars were compared with genetic resource accessions obtained from gene banks and collected in collection missions. One of the most productive collection missions in Latvia was a joint project with a rural youth organization `4-H`, where rural youth was encouraged to collect faba beans grown in their family gardens over decades and to submit a handful of seeds to Pūre Horticultural Research Centre together with a short description. During 2014, with the help of `4-H`, 48 accessions were collected. A sub-set of these were evaluated during 2015 in field trials together with gene bank accessions and local old cultivars.

Here, the preliminary evaluation results of productivity traits of bean genetic resource collections, obtained over two years at the Pūre Horticultural Research Centre, the Institute of Agricultural Resources and Economics, and the Estonian Crop Research Institute are presented. The yield parameters revealed that the average number of pods per node varied between 0.9 and 2.4 broad beans and field beans respectively, and differed between genotypes and locations. The highest number of pods per node were found in the cultivars `Favel`, 'Gerd', Fb-2939, and 'Gubbestad' for broad beans and in 'Bauska', 'Lövånger', 'Lielplatones', `Valmiera`, VF_013 and 'Gloria' for field beans. The cultivars with the highest seed weight per plant were `VF_001`, `VF_003`, 'VF_004', 'VF_005', 'VF_009' and 'Gerd' in broad beans (50.8 g, on average) and for 'VF_007' and 'Lielplatones' in field beans (36.9 g, on average). Additionally, the 100 seed weight ranged from 70 to 147 g for broad beans and from 32 to 83 g for field beans. The highest number of seeds per pod in Pūre was obtained from 'VF_013' (4.1), 'Polli' (3.7), 'Favel' (3.8) and 'VF_002' (3.7).

Overall, it was found that the most promising cultivars were the broad bean accession 'VF_001', which showed the highest yield of 35.8 g per plant with relatively high resistance to pathogens, and the field bean accessions 'VF_007' (yield of 36.9 g per plant), 'Gubbestad' (31.4 g per plant), 'Lielplatones' (28.7 g per plant).

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Propagation of honeybush (*Cyclopia subternata*)

Gugu S. Mabizela[a,b], Jenifer Koen[a,b], Retha M. Slabbert[a] and Cecilia Bester[b]

[a] *Horticulture Department, Tshwane University of Technology, South Africa*
[b] *Cultivar Development and Horticulture, ARC Infruitec Nietvoorbij, South Africa*

mabizelag@arc.agric.za

Honeybush (Genus: *Cyclopia* Vent.; Family: Fabaceae) is a medicinal plant endemic to the South African fynbos biome that is used to make a herbal infusion known as honeybush tea. The tea is thought to have health benefits and is characterised as caffeine-free and having a low tannin content. Previously, medicinal plants have not been fully researched, partly because of the lack of knowledge of their existence. Recently, the importance of the health benefits of medicinal plants such as honeybush have been realised. However, due to the unsustainable wild harvesting of honeybush, the availability of plant material for processing has been limited. Thus, propagation of honeybush through seed and cuttings became necessary due to the rapid growth of the industry and the demand for more plant material. The objectives of this study were to determine the difference in germination of *C. subternata* dimorphic seeds (green and brown) and the rooting potential of stem cuttings. The seeds were divided by colour and their germination responses to several treatments (scarification, stratification, seed age and germination temperature) were evaluated. The highest germination percentage of scarified seeds was obtained after 3 weeks of cold stratification at 2°C and incubation at 15°C. Brown seeds stored for three years gave a significantly higher germination percentage at 8.44% than seeds stored for one year only at 1.78%. In non-treated seeds, brown seeds had a better germination percentage at 5.04% than green seeds at 1.19%. In treated seeds, green seeds had a better germination percentage than brown seeds (81% and 10.4% respectively for scarified seeds) and (33.07% and 6.67% respectively for stratified seeds). Cuttings of four clones were treated with three growth regulators and planted in three different growth media in a randomised block design. Different rooting media and different concentrations of growth regulators significantly affected the rooting of stem cuttings. The highest survival rate (87.8% and 87.7%), rooting percentage (69% and 68.3%) and root length (151.3 mm and 156.6 mm) were recorded with Seradix® B2 and Seradix® B3 growth regulators, respectively, while cuttings treated with Dip & Root™ growth regulator had significantly lower survival rate, rooting percentage and root length (84.1%, 63.9% and 128.5 mm, respectively). Cuttings propagated in a sand mixed with bark or in a peat-polystyrene mix had higher rooting and survival percentages and longer roots than the cuttings propagated in a peat mix. These results on propagation can now be implemented into the honeybush breeding programme, and also used to formulate guidelines for farmers on how to propagate honeybush successfully.

Featuring diversity of Portuguese common beans (*Phaseolus vulgaris* L.) through the study of phenolic composition

Elsa Mecha[a], Geoffrey Istas[b], Rodrigo Feliciano[b], Ana Rodriguez-Mateos[b], Maria Eduardo Figueira[c], Maria Carlota Vaz Patto[a] and Maria do Rosário Bronze[a,c,d]

[a]*Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Portugal*
[b]*Division of Cardiology, Pulmonology, and Vascular Medicine, Medical Faculty, University of Düsseldorf, Germany*
[c]*Faculdade de Farmácia, Universidade de Lisboa, Portugal*
[d]*Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal*

emecha@itqb.unl.pt

Common bean (*Phaseolus vulgaris* L.) represent one of the most widely distributed grain legumes in the world. During centuries small-scale farmers took advantage of common bean genetic diversity to select varieties adapted to different environmental conditions. Such diversity has a valuable economic impact for local communities, allowing an improvement in their nutritional and health status. The benefits attributed to common beans have been related with nutrient content, but also with bioactive compounds (e.g phenolic compounds) content. Phenolic compounds are secondary plant metabolites with antioxidant properties responsible for increasing plant resistance to adverse environmental conditions and preventing chronic diseases (e.g. cancer, diabetes, cardiovascular diseases) in common bean consumers.

Although poorly characterized, Portugal has a huge genetic diversity of common bean due to several centuries of their local cultivation. Under the framework of a Portuguese Project (BEGEQA - Exploiting Bean Genetics for food Quality and Attractiveness Innovation) we analysed the phenolic content and profile of 170 different varieties of Portuguese common bean. Varieties were cropped using a randomized block design in two different environments. Phenolic compounds were analysed using a Q-TOF-LC-MS system, after extracted from whole flours with a methanol: water (60:40, v/v) solution. Quantitative differences were established between varieties using multivariate data analysis (Principal Component Analysis followed by cluster analysis). The understudied common bean varieties revealed interesting qualitative and quantitative differences in phenolic composition.

The study contributed to elucidate the impact of common bean genetic diversity in the quantified phenolic compounds, as well as to identify interesting sources of high phenolic compounds which can be useful to improve common bean phenolic composition in future breeding programmes.

Assessment of genetic diversity among oilseed rape (*Brassica napus* L.) cultivars using single locus microsatellite markers

Katarzyna Mikolajczyk[a], Joanna Nowakowska[a], Jan Bocianowski[b], Alina Liersch[a], Wieslawa Poplawska[a], Stanislaw Spasibionek[a], Teresa Cegielska-Taras[a] and Iwona Bartkowiak-Broda[a]

[a] *Plant Breeding and Acclimatization Institute-NRI, Research Division in Poznan, Poland*
[b]*Department of Mathematical and Statistical Methods, University of Life Sciences in Poznan, Poland*

kamik@nico.ihar.poznan.pl

The aim of this work is to analyze the molecular characteristics of a population including oilseed rape cultivars and breeding lines collected at the Plant Breeding and Acclimatization Institute-NRI in Poznan, Poland, and comprising double-low and traditional cultivars, F_1 *ogura* CMS hybrids and parental components, breeding lines with changed fatty acid composition - high oleic and low linolenic forms in addition to new *B. napus* genotypes obtained by resynthesis. A set of single locus microsatellites (STR, Short Tandem Repeats) was chosen for analysis, as, first of all, they are specific either for the *B. napus* A or C genomes, thus enabling the preliminary study of recombinant genomes rearrangements. Moreover, STR loci are universal, species-specific, evenly distributed throughout the genome and also the STR assay is easy to perform, repeatable and relatively cheap. CTAB extracted total DNA will be PCR amplified using STR loci specific primer pairs, labeled fluorescently by 'M13 tailing' method. Then, the amplified loci resolved by capillary electrophoresis will be analyzed and Nei and Li genetic similarity coefficients will be estimated followed by constructing of the UPGMA dendrogram to show genetic relationships among the surveyed collection with respect to previously obtained dendrogram using AFLP technique. The obtained results will be applied for further genetic analyses and association studies.

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Phytochemical study of some native ajowan ecotypes from Iran

Seyedeh Maryam Mirzahosseini[a], **Seyed Ahmad Sadat Noori**[a], Yaghoub Amanzadeh[b], Majid Ghorbani Javid[a] and Mehdi Soltani Howyzeh[a]

[a] *Department of Agronomy and Plant Breeding, College of Abouraihan, University of Tehran, Iran*
[b] *Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Science, Iran*

noori@ut.ac.ir

This study was carried out to determine the appropriate ecotype for efficiency of essential oil and the amount of thymol, γ-terpinene and p-cymene in the essential oil. Twenty-three ecotypes of ajowan seeds from the gene bank of the Research Institute of Forests and Rangelands (RIFR) of Iran were prepared and planted in the farm of the College of Abouraihan, University of Tehran, Pakdasht, Tehran, Iran. After harvest, essential oils were extracted from seeds by hydrodistillation, and their chemical compounds were studied by GC-GC/MS. Based on the results, the amount of essential oils ranged form 2.5 % in 'Arak' and 6.1% in 'Sarbisheh' ecotypes. In all samples, 95% of the essential oil compounds were identified, with three main compounds: p-cymene, γ-terpinene and thymol. Thymol, the main compound of ajowan essential oil, varied from 34% to 55%.The highest and lowest percentage of thymol were for the 'Shahedieh' and 'Birjand' ecotypes, respectively. The highest and lowest thymol yields were for the 'Ardabil and 'Sarbisheh' ecotypes, respectively. There was a significant negative correlation between sabinene and beta-pinene, sabinene and alpha-pinene, γ-terpinene and thymol. Factor analysis also identified 3 factors accounting for a total of 73.43 percent of the total variance. Cluster analysis divided the ecotypes into three categories and F Belle's test confirmed the significant influence of all oil compounds on clustering except oil content and p-cymene content.

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Interspecific hybridizations in cool season food legumes

Duygu Sari[a] and **Cengiz Toker** [a]

[a] *Department of Field Crops, Akdeniz University, Turkey*

toker@akdeniz.edu.tr

Alien gene transfer from wild relatives has led to important improvement in crop plants, since cultivated genotypes lack adequate sources of resistance to abiotic and biotic stresses. Wild species are rich resources of useful alien genes which are not available in the cultivated species. These genes are associated with resistance to diseases and insects, tolerance to drought, salinity, low and high temperature and other abiotic stresses as well as quality traits. Cool season food legumes including chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* L.), pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.) are exposed to these stresses. Therefore, low yield of the cultivated genotypes is discussed frequently. Based on the degree of sexual compatibility, the source of diversity or "gene pool" used for the improvement of a given species was classified into the primary, secondary, and tertiary gene pools. The primary gene pool of chickpea consists of *C. arietinum*, *C. echinospermum*, and *C. reticulatum*. These species are crossable with *C. arietinum*, but with reduced fertility of the resulting hybrids and progenies; nevertheless, all species are cross-compatible with the cultivated species and do not need in vitro interventions to produce hybrids. The secondary gene pool consists of *C. pinnatifidum, C. bijigum,* and *C. judaicum*. Hybrids between *C. arietinum* X *C. judaicum*, *C. arietinum* X *C. judaicum*, and *C. arietinum* X *C. bijigum* are possible via embryo rescue and tissue culture techniques. *C. yamashitae, C. chrossanicum*, *C. cuneatum*, and perennial wild *Cicer* species are considered to be in the tertiary gene pool as none of the species of this group are known to cross readily with the cultivated species and produce mature seeds. Introgression of desirable genes from wild taxa to the cultivated species might help the flow of useful genes into cultivated lentil. However, there has been limited success in transferring biotic and abiotic stress resistance from wild *Lens* to cultivated lentil, mainly because of postzygotic barriers. The *Lens* species within the primary gene pool are readily intercrossed and produce almost fully fertile progenies. *L. culinaris* subsp. *orientalis* is readily crossable with the cultivated lentil and crosses between cultivated lentil and *L. culinaris* subsp. *odemensis* and also, *L. culinaris* subsp. *tomentosus* produce partially fertile hybrids. However, cultivated lentil crossed with *L. ervoides* or *L. nigricans* causes pod abortion. Embryo rescue techniques make possible the development of interspecific hybrids between the cultivated lentil and *L. ervoides* and *L. nigricans*. The primary gene pool of the genus *Pisum* consists of the Pisum *sativus/elatius* complex, although it is difficult to specify concisely because of the fertility barriers. A secondary gene pool generally extends to the other species in the genus, *P. fulvum* and *P. sativum* subsp. *abyssinicum*. The tertiary gene pool currently consists of *Vavilovia formosa* (Stev.) Fed. (= *Pisum formosum* (Steven) Alef., *Pisum aucheri* Jaub. & Spach.). As no wild faba bean has ever been found and because *V. faba* does not cross with other *Vicia* species, the wild ancestor of *V. faba* remains unknown. Using alien genes for introgression of these genes from wild taxa to cultivated species is very critical to develop stress-related resistance genotypes via crosses between wild types and cultivars.

New canola (*Brassica napus* L.) mutant lines with similar phenotypes and different fatty acid composition

Anna V. Shirokova[a], Valentina T. Volovik[b], Larisa M. Korovina[b], Anatoliy V. Zhukov[c], Vasiliy P. Pchelkin[c], Vladimir D. Tsydendambaev[c], Remir G. Kostyanovskiy[d], Oleg N. Krutius[d], Gul'nara K. Kadorkina[d], Alexandra V. Amosova[e], Olga V. Muravenko[e] and Nina A. Dokudovskaya[b]

[a]*Koltzov Institute of Developmental Biology, Russian Academy of Sciences, Russia*
[b]*All-Russian Williams Fodder Research Institute, Russia*
[c]*K.A. Timiryazev Institute of Plant Physiology, Russian Academy of Sciences, Russia*
[d]*N.N.Semenov Institute of Chemical Physics Russian Academy of Sciences, Russia*
[e]*Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Russia*

glandularia@yahoo.com

Canola (*Brassica napus* L.) is cultivated as a source of valuable vegetable oil with high content of polyunsaturated fatty acids (FA) such as oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$). In the present study, for improvement of morphological traits of canola cv. 'Vikros' seeds with oil content at least 40-45% and composition consisting 60% of C18:1, 20% of C18:2 and 9% of C18:2) were treated with aqueous solutions of chemical mutagens for 16 hrs. The following concentrations (%) were used: ethyl methanesulfonate (EMS) - 0.02, 0.03, 0.04, 0.2, 0.3; dimethyl sulphate (DMS) - 0.02, 0.08, 0.06 and diethyl sulphate (DES) - 0.06, 0.05. Seeds soaked in water were used as a control. Plants were grown at the Kropotovo Field Station (Moscow region). The flower buds were isolated for producing seeds of M_2 and the following generations. The oil was extracted with hexane from seeds, and its composition was determined with a gas chromatograph by standard technique. In M_2 and M_3 generations, 33 variations of morphological traits were found, and then the forms with similar phenotypes were selected for producing lines. They included plants with a compact growing habit, lodging-resistant short-growing forms, and the plants with long (until 10cm) or wide (0,7cm) shatter-resistant siliqua. In the lines of M_5-M_6 with similar phenotypes, fatty acid composition was analyzed for selecting the lines with high oil content in the seeds and a well-balanced FA ratio. It was found that oil content ranged from 35 to 55%. In most lines, the variability of FA amount increased due to "short" FA. For instance, from 0.2 to 2.4% of one of the "short" fatty acids C8:0 (caprylic acid) was detected in "long siliqua" line 83-623-24 (0.02% DMS). At the same time, almost the equal content of C18:1 (~65%) was found in lines 83-623-24 and 78-2-2-27 (0.04% EMS). In case of using 0.03% EMS, only 57% of C18:1 was revealed, but the content of C18:2 increased to ~24%. "Wide siliqua" lines differed slightly in the content of oil and main FAs. In lines 79-1517-2 (0.03% EMS) and 80-535-13 (0.02% EMS), about 63% of C18:1 was found and the oil content ranged from 46 to 50%. However, in some lines (0.03% EMS), 0.1 - 0.6% of erucic acid was detected. In some short-growing lines (0.03% EMS) with a compact habit and compact arrangement of siliqua, the increased content of C18:2 (up to 25 %) was detected, though 63% of C18:1 and only ~ 6% of C18:3 were present. Thus, the new mutant lines having constant heritable morphological traits differed not only in the FA contents, but also in their qualitative composition.

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Beans with benefits: Integrating improved mungbean as a catch crop into the dryland systems of South and Central Asia

Roland Schafleitner[a], Ramakrishnan Nair[b], Shernabi Khan[c], Bakhodir Kuziev[d], Pepijn Schreinemachers[a], Thomas Hilger[e] and **Svein O. Solberg**[a]

[a] *AVRDC – The World Vegetable Center, Headquarters, Taiwan*
[b] *AVRDC – The World Vegetable Center South Asia, India*
[c] *AVRDC – The World Vegetable Center, Pakistan*
[d] *AVRDC – The World Vegetable Center, Uzbekistan*
[e] *Hohenheim University, Institute of Agricultural Sciences in the Tropics, Germany*

svein.solberg@worldveg.org

Mungbean (*Vigna radiata*) is a popular pulse crop, producing protein-rich food and nitrogen-rich residues. Dryland cultivation of mungbean in South and Central Asia is constrained by a shortage of water, high salinity soils, yellow mosaic disease (begomovirus) and storage pests like bruchids (*Bruchidae sp*.). The "Beans with Benefits" project aims to increase mungbean cultivation in these regions. The project has four components: 1) identify salt tolerant, bruchid resistant and *Mungbean yellow mosaic virus* (MYMV) resistant germplasm; 2) share this germplasm with breeders and researchers; 3) develop production technologies; and 4) capacity building. Accessions from the AVRDC genebank and from other genetic sources are screened to identify tolerant and resistant germplasm. In an early stage of the project, resistance to MYMV was mapped on top of chromosome 5, responsible for almost 50% of the variation in resistance observed. A nucleotide marker tightly associated with the resistance locus was converted to a PCR-based marker and made available for breeders. In addition, three minor resistance loci were detected. Resistance against bruchids has been found in wild mungbean and in two cultivated accessions. Resistance loci were successfully mapped and three major loci were validated for both resistance sources. Markers at the resistance loci on chromosomes 3 and 4 predicted resistance and susceptibility correctly at 98.5% for both resistance sources TC1966 and V2802, while markers along the resistance locus on chromosome 5 correctly predicted the resistance phenotype at 100%. Thirteen PCR-based markers to select for the three loci have been shared with breeders for marker-assisted selection of bruchid resistant genotypes. Thirty bruchid resistant (F_7) lines have been selected for distribution to partners in two target countries. Additionally, 30 lines with resistance to powdery mildew and potential resistance to MYMV were identified for distribution to partners. In Pakistan, the first ever mungbean learning alliance has been established, consisting of researchers, farmers, input suppliers, processors, and extension workers. Pulses are important cash crops in Pakistan but are sensitive to environmental changes and are costly due to manual harvesting. Hence, crop improvement suitable for mechanical harvesting and resistance to bruchid and MYMV is emphasized. Heat tolerance, sprouting resistance and bug control are other important issues. The AVRDC mini-core collection has been multiplied successfully in both countries. In Uzbekistan, mungbean is a well-known legume but there is a need for new sources of genes to develop extra-early maturing, drought- salt- and heat-tolerant varieties. Every year after the winter grain harvest, 1.5 million hectares are available for secondary cultivation. For the first time, a comprehensive study on mungbean has been conducted in the country with a series of field trials. Identification of technologies for increasing soil fertility and crop productivity are being developed. This includes greenhouse and growth chamber trials examining temperature and drought impact on production, plant growth promoters as affected by salinity, decomposition of mungbean residues, and uptake of nutrients by follower crops.

Phenotypic variability of resynthesized oilseed rape (*Brassica napus* L.)

Katarzyna Sosnowska, Laurencja Szała and **Teresa Cegielska-Taras**

Plant Breeding and Acclimatization Institute, National Research Institute, Department of Genetics and Breeding of Oilseed Crops, Poznan, Poland

tceg@nico.ihar.poznan.pl

The level of genetic diversity in double-low genotypes of oilseed rape is relatively low. This is caused above all by intensive selection of genotypes in terms of two features associated: with improving oil, through eliminating erucic acid and with improving the meal, through the reduction of glucosinolates content. Today, oilseed rape breeders are seeking genetic diversity in their breeding programs. A particularly successful method used to create novel genetic variation is wide hybridization in a special resynthesis of *B. napus* from ancestral species *B. oleracea* and *B. rapa.* In the present study resynthesized oilseed rape was obtained as a result of reciprocal crosses between different *B. rapa* and *B. oleracea* subspecies using two methods: 1). *in vivo* pollination and then *in vitro* culture of isolated embryos in the early stage of their development (embryo rescue culture), 2). *in vitro* placental pollination and then embryo rescue culture. Plants obtained from interspecific crossing were investigated for nuclear DNA content via flow cytometry to confirm their hybrid genotype. These plants were treated with colchicine in order to obtain amphidiploid *B. napus* plants. These resynthesized (RS) plants were assessed for morphological variation by comparing selected characteristics such as leaf shape and flower size, chlorophyll content in leaves as well as pollen fertillity due seed formation. Very large differences in studied traits have been found among novel RS lines, especially in the seed development. Probably it is the reason that *B. rapa* and *B.oleracea* often display self-incompatibility although natural *B. napus* is a facultative outcrossing species with a high degree of self-pollination. Biochemical analysis revealed that large parts of RS seed characterized with high level of erucic acid and glucosinolates. Only few RS lines possessed seeds with zero erucic acid but high level of glucosinolates.

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Genetic, proteomic and physiological background for breeding common bean for abiotic stress resistance

Jelka Šuštar-Vozlič[a], Marko Maras[a], Mateja Zupin[a], Aleš Sedlar[a], Tanja Zadražnik[a], Jaka Razinger[a], Marjetka Kidrič[b], Dominik Vodnik [c], Barbara Pipan[a] and Vladimir Meglič[c]

[a] *Agricultural Institute of Slovenia, Slovenia*
[b] *Institute Jožef Stefan, Slovenia*
[c] *Biotechnical Faculty, Agronomy Department, Slovenia*

jelka.vozlic@kis.si

Various abiotic factors affect plant growth and crop productivity. Drought is one of the main abiotic stresses for common bean (*Phaseolus vulgaris* L.), causing considerable loss of yield in many regions worldwide. Development of cultivars tolerant to drought stress has therefore become one of the primary goals of common bean breeding programs. Our research work in the last decade has been oriented in several directions in order to obtain a deeper insight into the molecular and morpho-physiological adaptation of this crop to drought. A differential gene expression study, conducted on leaves of eight genotypes at different levels of dehydration, revealed that drought induced alteration of 15 drought-responsive transcripts, eight being increased and seven decreased. Two-dimensional differential in-gel electrophoresis, in combination with mass spectrometry, was used to identify drought-responsive proteins in leaves of two cultivars differing in their response to drought. The identified proteins were grouped in functional categories, including metabolic proteins, cell defence/stress proteins, proteins involved in photosynthesis and proteins with unknown functions. Analysis of the proteome of the stem of a more resistant cultivar, using in-gel stable isotope labelling, revealed that proteins differentially expressed under drought stress also belong to several functional groups involved mainly in energy metabolism, photosynthesis, in response to reactive oxygen species, stress and proteolysis. A proteolytic enzyme from common bean leaves, whose activity was influenced by water deficit, has been isolated and characterized at the protein and gene levels. It is a new plant subtilisin-like protease, whose activity in leaves of a moderately sensitive cultivar increased under drought, with no changes in its gene expression. The population of 80 recombinant inbred lines (RILs) of the F8 generation, derived by a cross between a more and a less sensitive cultivar was used to develop a genetic map. Plants grown in the greenhouse were stressed by withholding irrigation at the flowering stage. Both parental lines and all RILs were checked for polymorphisms using DNA markers. In addition, physiological and morphological parameters that distinguish the two parental lines, such as water potential and photosynthetic parameters, grain and flower colour, days to flowering, seed yield and weight of seeds, were measured in RILs at different stages of drought. Out of 476 SSR and 256 AFLP markers tested, 127 were polymorphic and were further used to construct a genetic map. Markers were arranged into 11 linkage groups (LGs) from the common bean consensus map and two additional, unclassified LGs. QTL analysis revealed a linkage between markers and QTLs for days to flowering and single seed mass in LG1 and LG9 from the consensus map. These markers will be used in a breeding program aimed at improving drought tolerance in common bean cultivars.

Evaluation of resistance to ZYMV among selected orange cultivars *Cucurbita maxima*

Jiri Svoboda

Crop Research Institute, Division of Plant Health, Czech Republic

jiri.svo@vurv.cz

Zucchini yellow mosaic virus (ZYMV) causes considerable losses of cucurbitaceous vegetables grown nearly all over the world. Orange cultivars of *Cucurbita maxima* are greatly appreciated by consumers due their edible skin when cooked, longevity and high carotene content. Commonly planted orange cultivars are highly susceptible to ZYMV. Three pumpkin cultivars type 'Red Kuri' from South Africa and three trom the Czech Republic were selected for evaluating their resistance to the most virulent Czech strain ZYMV-H (GenBank Acc. No. DQ144054, Virus Collection of Crop Research Institute, Prague). Butternut squash (*Cucurbita moschata*) 'Menina 15', reported to be resistant to ZYMV, was also included in the study. Seeds of the tested cultivars were sown and young plants were mechanically inoculated with ZYMV-H. Their resistance was assessed four weeks later after the systemic infection occurred. Relative concentrations of ZYMV protein in the leaves of the inoculated plants were assessed on the basis of the virus titer determined by ELISA, while resistance was evaluated by comparing the virus protein relative concentration among the tested cultivars and observed symptoms. As expected, the butternut squash 'Menina 15' was immune, its leaves were virus free and the inoculated plants did not show any symptom of infection. The absence of the virus in leaves was proved by PCR molecular test as well as observations at the electron microscopy. African pumpkin 'Invincible' and 'Star 7026' were resistant and the virus multiplied in their plants at a low rate. The Czech pumpkins 'Grey Queen' and 'Blue Kuri' showed to be tolerant while 'Hokkaido' highly susceptible to ZYMV-H. The latter showed a leaf virus concentration four times higher than the resistant cultivars and displayed severe mosaic symptoms.

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Effect of *in vitro* and *in vivo* colchicine treatment on efficient production of doubled haploid plants of oilseed rape

Laurencja Szała, Marek Wójtowicz, Katarzyna Sosnowska and Teresa Cegielska-Taras

Plant Breeding and Acclimatization Institute – National Research Institute, Department of Genetics and Breeding of Oilseed Crops, Poznań, Poland

lszala@nico.ihar.poznan.pl

Development of doubled haploid (DH) plants through microspore culture is an important and efficient method of production of fully homozygous lines. This method has wide application in genetic and breeding programs. Haploid technology for *Brassica napus* is probably the most highly developed system among flowering plant species. Since microspore-derived plants originate from gametes, they have usually a single set of chromosomes, and in the vast majority they are haploids. In the case of oilseed rape, the rate of spontaneous chromosome doubling is low and range from 10% to 30% depending on the genotype. This number of diploids is unsatisfactory, therefore, the use of effective techniques for chromosome duplication such as application of antimitotic agents is necessary. Chromosome doubling agents can depolymerise microtubules and disrupt mitotic cell division (Hansen and Andersen 1996). Among agents promoting the production of DH plants (e.g., colchicine, oryzalin, trifluralin and amiprophos methyl), colchicine is the most commonly applied one because of its relative simplicity, water solubility and storage potential. Colchicine can be applied across several stages of the microspore culture process—from isolated microspores to the regenerated plants. The usual methods of chromosome doubling involve soaking roots or whole plants in a colchicine solution, culturing plantlets in colchicine-containing medium in the greenhouse or using colchicine in isolated microspore media (Mohammadi et al. 2011). The best stage to apply colchicine are freshly isolated microspores. The *in vitro* colchicine treatment of microspores does not cause any developmental delay and can improve induction of embryogenesis. However, sometimes the number of chromosomes has to be doubled in the plants by soaking the roots or shoots in a colchicine solution. Although these methods are laborious, time consuming and result in ploidy chimeras, they are used: i) to duplicate chromosomes in haploid plants developed from different explants subjected to mutagenesis or transformation and ii) for plants obtained through interspecific crosses for resynthesis of oilseed rape. The purpose of this study was to evaluate the effects of *in vitro* colchicine treatment on microspore culture and *in vivo* colchicine treatment of young plants and auxiliary shoots on the efficiency of diploidisation of different winter oilseed rape breeding materials.

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Recent molecular studies on development of sunflower genotypes with high oleic acid content

İlknur Tindas[a] and Sevgi Çalışkan[b]

[a]*Department of Agricultural Genetic Engineering, Faculty of Agricultural Sciences and Technologies, Niğde University, Niğde, Turkey*
[b]*Department of Plant Production and Technologies, Faculty of Agricultural Sciences and Technologies, Niğde University, Niğde, Turkey*

ilknur4888@hotmail.com

The sunflower, which is among the most important oil crops for markets, is composed of linoleic acid (C-18:2) and oleic acid (C-18:1). Development of new cultivars with high oil content is one of the main principals of sunflower breeding programs. The program includes the understanding of the genetic control of characters and development of marker-assisted selection programs concerning oil percentage in grain, grain yield and agronomic traits. QTL studies on agronomic traits, such as oil percentage of grain, indicate that molecular markers are related to oil characteristics and quantitative genetics analyses. Besides, genetic modifications of high fatty acid composition in sunflower show that sunflower is one of the most promising crops for genetic alteration of oil quality. In the studies to develop genotypes with high-oleic acid content by incorporating *Ol+tph1* gene into sunflower, oleic acid content was increased over 90%. To develop higher fatty acid composition and seed oil content, other genes may be incorporated into sunflower lines in future studies. Recent molecular studies associated with high oleic acid traits in sunflower genotypes will be discussed in this presentation.

Landrace common bean (*Phaseolus vulgaris* L.) genotypes collected from Western Mediterranean region of Turkey

Kamile Ulukapı[a], Köksal Aydınşakir[b] and Rana Kurum[b]

[a]*Akdeniz University, Vocational High School of Technical Sciences, Organic Agriculture Program, Turkey*
[b]*Bati Akdeniz Agricultural Research Institute Turkey*

kamileonal@akdeniz.edu.tr

Being one of the agricultural products to be commonly grown and consumed in the world, beans display a wide range of production and sort in Turkey though it is not a native land. Although the beans production is limited to some parts in Turkey, it is commonly cultivated for family consumption both at low and high-altitude territories. However, the demand towards the commercial seeds seems to be increase today, and this situation threatens the presence of landrace genotypes. The landrace genotypes, one of the genetic sources, are crucial for maintaining the genetic variability, food safety and breeding applications. From this point of view, the study has been conducted with the landrace genotypes to have been grown at some towns and villages of the provinces Antalya, Burdur and Isparta in Western-Mediterranean of Turkey between the years 2013 and 2014. In order to develop a gene pool and to make an beginning material for breeding studies, 125 landrace common bean genotypes adapted to both coastal line and highlands have been gathered, recorded and taken under protection with the detected locations.

Selection of white lupine genotypes for yield and tolerance to alkaline soils

Dimitrios N. Vlachostergios[a], Ioannis Divanes[b], Apostolos Fyntanis[c], Eleni Koseoglou[b] and Athanasios G. Mavromatis[c]

[a]*Industrial & Fodder Crops Institute, Hellenic Agricultural Organization-Demeter, Greece*
[b]*Plant Science & Rural Environment Department, University of Thessaly, Greece*
[c] *School of Agriculture, Aristotle University of Thessaloniki, Greece*

vlachostergios@gmail.com

White lupine (*Lupinus albus* L.) is a significant protein crop that is well adapted to dry areas and poor soils. White lupine's expansion is limited in many regions as it cannot be cultivated in soils with PH >7 and $CaCO_3$ > 2%. Thus, cultivation of lupine genotypes that can tolerate high PH values is considered the most economic and efficient solution. The objective of the current study was to evaluate white lupine landraces and advanced populations for yield and tolerance to alkaline soils and to develop an efficient breeding scheme to identify the most suitable genotypes. Experiments were established in two locations with different soil characteristics during two growing seasons (2014-2015). Location 1 (L_1) was considered as optimum environment (PH = 6.5, $CaCO_3$: 0.0) and Location 2 (L_2) as stress environment (PH = 8.1, $CaCO_3$: 2.6). In the first culture period, 27 entries (landraces and advanced populations) were evaluated for their yield potential under both locations in an RCBD with three replications. In L_1 mean yield ranged from 1.4 to 4.12 t ha^{-1}, while in L_2 yield ranged between 0.2-1.5 t ha^{-1}. A combined analysis was conducted and 13 entries that showed wide adaptability were selected as promising genetic material for further experimentation. The next year, single plants originating from the 13 entries were grown under an R-13 honeycomb design (Fasoulas and Fasoula, 1995) with 50 replications that was established in both locations (650 plants/location). Single plants were sown in a low plant density (1.2 plants·m^2) that is asserted to optimize genetic expression. Seed yield/plant along with pods/plant, seeds/pod, earliness, SPAD value/plant were assessed. The data were analyzed with a specific computer program for honeycomb designs (Mauromoustakos et al., 2006). Cross-over alterations were observed on the entry ranking between optimum and stress environments. Two entries with wide adaptability were detected. In L_1, mean seed yield/single plant of the trial was 52g, whereas mean entry yield ranged from 34 to 68g. The highest yielding single plant from each of the top seven entries was selected. In the stress environment (L_2), significant variability in phenotype expression and yield production was observed within entries. A high number (40%) of single plants didn't even reach maturity. Mean seed yield/single plant of the evaluated entries ranged from 1.8-4.6g, while the mean yield of the experiment was 3.32g. However, 32 single plants in the entire field with high seed yield (15-45g) were identified and among them the top six single plants were selected. Next year, the project will be continued with the progeny test of the selected plants in both optimum and stress environment. These preliminary results provide evidence of valuable genetic diversity among white lupine landraces and populations tested for simultaneous selection for yield and tolerance to alkaline soils.

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Crossbreeding of transgenic flax plants producing polyhydroxybutyrate and overexpressing the β-1,3-glucanase gene results in an increase in the polyamine content

Wioleta Wojtasik[a], Marta Preisner[a], Anna Kulma[a], Tadeusz Czuj[a], Katarzyna Namysł and Jan Szopa[a]

[a] *Department of Genetic Biochemistry, Faculty of Biotechnology, University of Wroclaw, Poland*

wioleta.wojtasik@uwr.edu.pl

Flax (*Linum usitatissimum*) is a valuable source of oil and fibers used in many industrial applications. Its cultivation is restricted by environmental stress factors, but the biggest crop losses worldwide are caused by *Fusarium* infection. In order to improve the resistance of flax plants to pathogen infection, transgenic flax called type B with overexpression of the β-1,3-glucanase gene was created. The advantage of these plants is increased content of polyamines, which also play a role in plant defense against pathogens and have antioxidant and antibacterial activity. Another transgenic flax type, called M, with overexpression of polyhydroxybutyrate (PHB) synthesis genes, was created in order to improve the biomechanical properties of flax fiber.

The main aim of this study was to generate a new flax type that produces polyhydroxybutyrate and has better resistance to pathogen infection, as well as increased content of polyamines. The development of such plants involved crossing two types of plants, type M and type B. The M type flax plant that produces polyhydroxybutyrate was crossed with type B flax plants overexpressing the β-1,3-glucanase gene. The new plants, called MB, were selected by verification of the expression of β-1,3-glucanase and chitinase genes using real-time PCR and by the assessment of plant seedlings' resistance to *Fusarium culmorum* and *F. oxysporum.* Lines of the new flax type MB which were characterized by an increase in expression of β-1,3-glucanase and chitinase genes were selected for further analysis. The analysis of the degree of infection of root and hypocotyls in flax seedlings after inoculation with pathogenic *F. oxysporum* sp. *linii* and *F. culmorum* mycelium confirmed that the MB plants were more resistant to pathogenic fungal strains. The next step was the assessment of the cell wall composition and contents of polyamine, phenolic compounds, sterols and fatty acids in the flax stem. Furthermore, an analysis of antioxidative properties of extract from flax stem was performed.

The analysis of amounts of cellulose and lignin, two key components of the cell wall polymers, showed no difference between the new flax type and the control. An immense increase in the polyamines content in the straw of MB plants was observed when compared to the control, in particular in a fraction of polyamine conjugated with phenolic compounds and in a fraction bound to the cell wall. Moreover, a 10-fold higher amount of spermidine was detected in the new flax straw. The analysis of the free phenolic compounds showed no significant changes, while in the case of the compounds bound to the cell wall their content was on average higher in the MB plants. The content of sterols in the straw was estimated by GC-MS, and no significant changes in their amount or in the amount of fatty acids in the straw were found.

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Parallel flash and poster presentations: Fruit, ornamentals and medicinal/industrial plants

Pyramiding of four QTLs for Fire blight resistance in apple

Ina Schlathölter[a], Simone Schütz[a], Markus Kellerhals[a] and **Andrea Patocchi**[a]

[a] *Institute for Plant Production Science IPS, Agroscope, Switzerland*

andrea.patocchi@agroscope.admin.ch

The apple cultivar 'Enterprise' (Co-op 30) is known for its high level of resistance to the bacterial disease fire blight (*Erwinia amylovora*). Carrying also the apple scab (*Venturia inaequalis*) resistance gene *Rvi6* this cultivar has reasonable sensorial and storage qualities. Therefore, 'Enterprise' is very interesting to use in breeding for new disease resistant cultivars. Recently, we identified in 'Enterprise' two QTLs for fire blight resistance on the linkage groups (LGs) 7 and 13 (Patocchi unpublished). In that experiment, when 'Enterprise' was artificially inoculated with *Erwinia amylovora*, it showed symptoms of fire blight on about 3% of the shoot length. On the other hand, the genotypes of the population used for the QTL mapping ('Gala' x 'Enterprise') carrying both favorable alleles showed fire blight symptoms on about one third of the shoot length. This result indicates that several minor effect QTL were not identified during the QTL mapping. In an earlier study, two minor QTLs for fire blight resistance in 'Florina' were mapped on the LG5 and 10 (Le Roux et al. 2010). While pyramiding these two minor QTLs with those of 'Enterprise' it might be possible to increase the level of fire blight resistance that can be obtained with the two 'Enterprise' QTLs only.
A cross between 'Florina' and 'Enterprise' was performed and the seedlings were tested with molecular markers (SNP and SSRs) associated to the four fire blight resistance QTLs. Out of all the possible combinations of the alleles of the four QTLs, seedlings belonging to 10 groups were chosen and inoculated with *Erwinia amylovora*. The results of the study of the different pyramids of fire blight QTLs will be presented and discussed.

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Sensory, chemical and molecular analysis of fresh strawberries (*Fragaria* × *ananassa* Duch.) over different cultivars in Western Greece reveals factors affecting eating quality

Konstantina Zeliou[a,c], **Vasilis Papasotiropoulos[b]**, Ioannis Manousopoulos[a], and Foteini N. Lamari[c].

[a]*Plant Protection Institute (Patras), Hellenic Agricultural Organization (HAO) DEMETER, Greece*
[b]*Laboratory of Plant Genetics, Department of Agricultural Technology, TEI of Western Greece, Greece*
[c]*Laboratory of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, University of Patras, Greece*

vpapasot@teimes.gr

Strawberry (*Fragaria* x *ananassa* Duch.) is a very important fruit, widely cultivated in the region of Western Greece. The aim of this study was to evaluate the strawberry cultivars' eating quality and also to understand the flavor components of the main genotypes cultivated in the region.

Three of the most common commercial cultivars, namely 'Camarosa', 'Fortuna' and 'Sabrina' were harvested in close dates within May 2015 from different localities and were evaluated for sensory properties by a panel of independent assessors. Ratings for overall taste, flavor and sweetness were below average for 'Fortuna', high for 'Sabrina' - a quite new cultivar - and above the average for 'Camarosa', the main strawberry cultivar grown in Greece. Firmness was higher in 'Sabrina', and similar for the other two cultivars, an evaluation confirmed by penetrometer measurement.

Chemical analysis was in agreement with the aforementioned sensory evaluation. Thus, in 'Sabrina' the soluble solid content (SSC) and the total sugars content (TSC) were found to be high, while the titratable acidity (TA) and the total ascorbic acid content were relatively low. On the other hand, the anthocyanic and polyphenolic content, as well as the antioxidant potential was found to be the highest in the 'Camarosa' cultivar.

qRT-PCR was performed on the same fruits, in order to examine the expression levels of *FaFAD1*, which likely controls synthesis of γ-decalactone, a key flavor volatile in strawberry that confers a peach note (Chambers et al. 2014; Sánchez-Sevilla et al., 2014). *GAPDH* was used as a normalizing gene. In 'Sabrina' cultivar, *FaFAD1* was expressed in the fruits from all localities sampled, while in 'Fortuna' only in the fruits from one of the sampled areas. No *FaFAD1* expression was detected in the 'Camarosa' samples that were tested in our study. Considering that *FaFAD1* expression is consistently present only in the most attractive cultivar, in terms of the sensory evaluation, it can be concluded that this gene may contributes to the taste of strawberries. However, *FaFAD1* gene is probably not the only characteristic that contributes to the overall sensory acceptance of the cultivar, since sweetness and acidity might also play an important role.

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Comparative transcriptomics of *Rosa corymbifera* Laxa roots with regard to replant disease

Carina Hieger[a], Elisabeth Wischnitzki[a], Eva M Sehr[a], Sebastian Baier[a], Angela Sessitsch[a] and Evelyn Hackl[a]

[a] *Health & Environment Dept., AIT Austrian Institute of Technology GmbH, Austria*

Carina.Hieger@ait.ac.at

When roses are repeatedly grown on sites where Rosaceae plants have previously been cultivated, they often show poor shoot and root development. This phenomenon of "rose replant disease" or "sick soil syndrome" is a significant problem in commercial rose cultivation. While the underlying mechanisms are not well known, scientific evidence shows that plant-soil-microbial interactions are a critical factor.

We have used a comparative transcriptomics approach to investigate the plant response to various soil conditions and different treatment regimes in rose replant disease. We extracted RNA from roots of *Rosa corymbifera* Laxa plants that were grown on differently "sick" soils and received different treatments, including autoclaving and amendment of compost and molasses fertilizers. RNA preparations were used for constructing cDNA libraries, which were sequenced using the Illumina Hiseq2000 technology. On average, about 50 million high quality reads were obtained per sample (Q30 and minimal length of 50 bp), resulting in a reference *de novo* assembly dataset. Comparative analysis of the root transcriptome allows us to comprehensively characterize the plant response under specific soil and treatment conditions. For instance, we found 251 genes significantly differentially expressed in roots of rose plants grown on a "sick" (10 years of rose cultivation) versus "fresh" (no previous rose cultivation) soil, encoding e.g. various transcription factors, transporter proteins, and proteins involved in different stress responses.

Our study results shall provide new insights into the mechanisms underlying rose replant disease and may implicate on novel treatments for overcoming adverse effects of replanting on plant health. Furthermore, an improved understanding of replant disease in roses may present a starting point for confronting other replant diseases.

Genotoxic effects of heavy metals on intergeneric *Helianthus* × *Echinacea* hybrid lines

Galya Petrova[a], Stefan Petrov[b], Roumiana Vassilevska-Ivanova[a] and Snezhanka Doncheva[a]

[a]*Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Bulgaria*
[b]*Institute of Molecular Biology "Roumen Tsanev", Bulgarian Academy of Sciences, Bulgaria*

galiaty@abv.bg

Heavy metals are among the most important sorts of contaminant in the environment. The toxic effect of heavy metals on plants depends on the type of metal, ion concentration, plant species and stage of plant growth, and usually results in growth inhibition, structure damage, a decline of physiological and biochemical activities, etc. [1]. Sunflower (*Helianthus annuus* L.) is considered one of the most important oil species, with a comparatively narrow genetic base. The wild relative *Helianthus* species represent a potential source of biotic and abiotic stress resistance genes. The present work is a part of a research program for production and evaluation of new intergeneric hybrids for transferring desirable traits from wild relatives to cultivated sunflower lines. In our previous study, new genetically stable hybrid lines between cultivated *Helianthus annuus* and *Echinacea purpurea* L., were developed by hybridization technique [2]. Recent data put in evidence that Inter Simple Sequence Repeat (ISSR) analysis is an applicable assay for the detection of genotoxicity caused by environmental pollutants [3, 4]. Extending the previous analyses on the total antioxidant capacity and morphological characteristics of established hybrids, we have performed ISSR analysis to detect changes occurring in the DNA profiles of *H. annuus x E. purpurea* after lead (Pb) and zinc (Zn) treatment at selected concentrations. Different polymorphic bands were observed, due to the dose dependent variations in band intensity, loss of normal bands and appearance of new bands. The obtained results indicate that the induced changes in the genomic profiles of both parents (*H. annuus* and *E. purpurea*) used in the present study as controls were higher as compared to hybrid lines at the same concentrations of heavy metals. Our finding could open up new possibilities for identification of target genes for specific genotoxic agents and design of new approaches to risk assessment.

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Ectopic expression of AHL24 in chrysanthemum plants delayed senescence and enhanced stress tolerance

Ji Hyun Kim[1], **Yeo Jin Youn[1]**, Dong Hee Lee[2] and Nam-In Hyung[1]

[1]*Department of Plant and Food Sciences, Sangmyung University, Cheonan, Rep. of Korea*
[2]*Genomine Advanced Biotechnology Research Institute, Genomine, Inc., Pohang, Rep. of Korea*

yjyoun01@gmail.com

AHL24 (*AT-hook motif nuclear localized protein*) gene under the control of stress inducible SEN1 promoter was introduced into leaf explants of chrysanthemum (*Chrysanthemum morifolium*) cv. Jinba via *Agrobacterium tumefaciens* LBA4404-mediated transformation. Sixteen independent transgenic plants were generated. The *AHL24* expression assay of the transgenic plants showed that there was a dramatic increase in transcript expression during the senescence stage. Comparison of the transcript level in different plant parts revealed that the transcript level was higher in the leaf and stem than in the flower and root. In the dark-induced senescence assay, senescence was delayed in transgenic plants, especially in leaves, compared with that in the WT plant. The *AHL24* transgenic plants showed a higher tolerance than the WT plant to the experimental stress conditions, such as the H_2O_2-induced oxidative and salt stresses, respectively. Senescence of the leaf and flower of cut flowers of *AHL24* transgenics kept in a growth chamber at 12℃ was delayed relative to that of the WT plant. These results demonstrate that a relatively higher expression of *AHL24* in the regenerated transgenic chrysanthemum plants conferred a longer delay in senescence and a greater tolerance to stress. Based on these results, we suggest that *AHL24*-expressing chrysanthemum plants could be used in plant breeding programs to develop genetically modified cultivars with delayed senescence and enhanced stress tolerance.

Towards *Silybum marianum*'s domestication: establishment and screening of a mutagenized population

Tommaso Martinelli

Centro di ricerca per le colture industriali, Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA-CIN), Italy

tommaso.martinelli@crea.gov.it

Silybum marianum (L.) Gaertn. (Asteraceae; common name: milk thistle) is a diploid, autogamous and annual plant species native to the Mediterranean area. At present, *S. marianum* is cultivated as a medicinal plant in Eastern Europe as well as in Asia. Medicinal properties of *S. marianum* are determined by its ability to accumulate the complex of bioactive flavonolignans referred to as silymarin in the fruit coat. Silymarin is among the top-selling herbal products in the U.S. and in other markets. Besides silymarin, the fruit also contains valuable products such as oil and proteins. From an agronomic perspective, the species is characterized by significant fruit and biomass yields, and has other possible non-medical applications (Andrzejewska *et al.* 2015). Despite the increasing interest in *S. marianum* as a multipurpose crop and its economic importance as an officinal species, the plant is still marked by traits that are typical of non domesticated plants: fruit shedding at maturity, asynchronous flowering, spiny leaves, variable productivity and quality. Based on this, a mutagenesis programme was implemented in order to: 1) reduce/eliminate fruit shedding at maturity (SHE phenotype), 2) modify fruit oil fatty acid profile (FAP phenotype).
S. marianum fruits were treated with ethyl methanesulfonate (EMS; 36 mM for 16 h at 22°C; 7.8 solution to fruit ratio, w/w). Five thousand plants were sown under field condition in single rows (M1 population). The central flower head of the 2239 plants that reached maturity was harvested. The M2 population was obtained using single seed descent approach and 23 plants with putative SHE phenotype were identified. The further field observation of the M3 SHE families allowed to confirm that two families show reduced fruit shedding. With regard to the FAP phenotype, the M2 fruits from all the 2239 harvested plants were analysed in pools of 60 fruits per plant for fatty acid profile (GC analysis). The analysis of the population box-plots for the single fatty acids highlighted 12 outliers with FAP phenotype. These 12 putative FAP families were sown in the field and M3 fruits were analysed. The results clearly showed two segregating families with codominant and recessive mutations, respectively. The first family displayed high oleic acid content with an average fruit content of 68.8 ±0.92% (±SE) in homozygous plants, the second mutant family had high stearic acid content with an average of 16.7 ±0.70% (±SE). The above-mentioned values are 1.55 and 2.17 folds higher than the control plants, respectively.
This mutation breeding approach allowed to identify a high oleic and a high stearic *S. marianum* mutant lines in three years. Further field observation are required in order to better evaluate the two lines with reduced fruit shedding at maturity. These *S. marianum* mutants will constitute the starting material for the further development of new varieties suitable for the production of biomasses and useful biomolecules in the Mediterranean area.

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Towards the development of a sterile chamomile variety (*Matricaria recutita* L.) using breeding, molecular and genomic tools

Lars-Gernot Otto[a], Andreas Plescher[b], Marlis Sonnenschein[b], Bettina Faehnrich[d], Chlodwig Franz[d] and Timothy F. Sharbel[a, c]

[a] *Apomixis Research Group, Institute for Plant Genetics and Crop Plant Research (IPK), Germany*
[b] *PHARMAPLANT Arznei- und Gewürzpflanzen Forschungs- und Saatzucht GmbH, Artern, Germany*
[c] *Global Institute for Food Security (GIFS), University of Saskatchewan, Saskatoon, Canada*
[d] *WG Functional Plant Compounds, University of Veterinary Medicine, Wien, Austria*

ottolg@web.de

German chamomile (*Matricaria recutita* L.) is an important medicinal plant from which flower heads containing the medicinal compounds are harvested. Since chamomile is quite resistant to herbicides and seeds can germinate after 10 to 15 years of dormancy, new fields for cultivation are difficult to gain, thus inhibiting crop rotation and causing problems like disease accumulation.
A sterile chamomile variety could overcome these problems, similar to triploid F1-hybrids in many fruit and ornamental crop plants (e.g. grape, banana, marigold). However, to efficiently produce triploids by crossing, selfing must be prevented, using genic (nuclear) male sterility, cytoplasmic-genetic male sterility (CMS) or self-incompatibility.
Triploid chamomile plants were (1) identified in a mixed di- and tetraploid population, and (2) generated by directed interploid crosses. All triploids showed high degrees of sterility. Genome wide genetic diversity of *M. recutita* was evaluated with GBS-SNP to select crossing partners for the potential exploitation of heterosis and CMS. To identify candidate factors for male sterility, genome-wide gene expression patterns between male sterile ray florets and male fertile disc florets were contrasted using microarrays.

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Comparing the efficacy of polyester tents and isolation chambers for hybridisation in *Miscanthus*

Charlotte Hayes[a], **Daljit S. Virk**[b] and Hannah Senior[c]

[a]*Institute of Biological, Environmental and Rural Sciences (IBERS), Aberystwyth University, Aberystwyth, Ceredigion, Wales, SY23 3DA UK*
[b]*School of Environment, Natural Resources and Geography (SENRGy), Bangor University, Bangor, Gwynedd, Wales LL57 2UW UK*
[c]*PBS International, Salter Road, Scarborough, YO11 3UP, UK*

d.s.virk@bangor.ac.uk

Plant breeders of grasses and forage crops often use tents or isolation chambers for making paired or poly-crosses for breeding new varieties. When tents made from fabrics are used the material needs to have pore sizes small enough to exclude contamination from foreign pollen. It is also important that the tent allows for temperature, humidity and gas exchange to be similar to the ambient environment as well as having strength, water and wind for optimal seed setting and development. Nonetheless, not much attention has been hitherto placed on testing the role of pollination tent choice in producing healthier seeds in higher quantity in comparison to isolation chambers.

We assessed the effect of climatic conditions on quantity of seed set following 16 crosses among *Miscanthus* species under four micro-climatic environments that were created by locating a tent made from a nonwoven polyester called duraweb® and isolation chambers in the glasshouse and in external climatic conditions. A Venlo glasshouse used for the tent crosses had temperature, lighting and irrigation controls. The isolation chamber is made from plexiglass, and air is drawn in through a pump to maintain a positive pressure, the intake air being filtered to remove pollen. duraweb® is a proprietary material created by bonding randomly laid polyester fibres. The fabric of the tent is breathable, allowing air and moisture to pass through but halts the unwanted pollen having an average pore size (14 microns) smaller than *Miscanthus* pollen grains.

The mean values for total number of seeds and average number of seeds per head were consistently higher for duraweb® tents whether in the external or glasshouse conditions. However, the total number of heads was higher in external tent (48.00) followed by isolation chamber in glasshouse (41.80). The glasshouse tent produced 83 seeds per head which was significantly higher by 82% than the isolation chamber in glasshouse conditions.

The lower seed set, on average, in the isolation chambers when compared with the crossing tents was probably because of differences in humidity and temperature created by the different crossing environments. The temperature inside the crossing tent (7-27 °C) was lower and more controlled than in the isolation chamber (5-40 °C). The humidity cycled continuosly in the crossing tent, whereas in the isolation chamber it stayed high for a period of 20 days which might also have affected pollen viability. We conclude that the duraweb® tents provides an environment that is conductive for higher seed set in *Miscanthus*.

Preserving the Swiss pear diversity

Christina Kägi[a], Christian Eigenmann[a], Hanspeter Kreis[a], Carlos Miranda[b], Luis G. Santesteban[b], J. Bernardo Royo[b] and Markus Hardegger[a]

[a] *Genetic Resources and Technologies Unit, Federal Office for Agriculture FOAG, Switzerland*
[b] *Departamento Producción Agraria, Universidad Pública de Navarra, Spain*

genres@blw.admin.ch

Pear trees have a long tradition in Switzerland. Hundreds of local varieties evolved over the last centuries. However, the diversity is threatened, mainly by the susceptibility of pear against fireblight, but also by the low demand for cider pears. To preserve the genetic pool of Swiss pear for future generations, the extant pear diversity was collected in a concerted effort with various private and public organisations. Around 1300 accessions were characterized with 16 SSR marker, resulting in 840 different genetic groups. Based on the molecular data, a core collection will be built to facilitate a potential future use of the diversity for breeding programs. This core collection might be further characterized on a pomological and agronomic level.

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Seed longevity in tobacco – Studies on intraspecific diversity and genetic determination

Monika Agacka-Mołdoch[a,b], Manuela Nagel[a], Teresa Doroszewska[b], Fiona R. Hay[c], Ramsey S. Lewis[d] and **Andreas Börner[a]**

[a] *Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany*
[b] *Institute of Soil Science and Plant Cultivation, State Research Institute, Puławy, Poland*
[c] *International Rice Research Institute, Metro Manila, Philippines*
[d] *Crop Science Department, North Carolina State University, Raleigh, USA*

boerner@ipk-gatersleben.de

With the aim to gather information about seed longevity of the genus *Nicotiana*, the viability of accessions stored at 20°C, 0°C and -15°C/-18°C for up to 12, 33, and 38 years, respectively, at seed banks in Poland and Germany was investigated. Logistic regression analysis was used to model the proportion of seed lots (accessions) with germination >75%. Considering this threshold, seeds of tobacco can be successfully maintained under controlled ambient conditions (20°C; paper bags) for up to ten years. At a storage temperature of 0°C (glass jars) this period is extended to about 30 years whereas after 40 years of storage at a temperature of -15°C/-18°C (glass jars) about 60% of the accessions show germination percentages higher than 75%. As in other genera an intraspecific variation was noticeable. Therefore, a genetic study was initiated using an already genotyped mapping population consisting of 122 recombinant inbred lines derived from a cross between the cultivars 'Florida 301' and 'Hicks'. Four germination-related traits were investigated by examining seeds either untreated or after controlled deterioration (CD): total germination (%), normal germination (%), time to reach 50% of total germination (h), and the area under the curve after 200 hours of germination. In total, four genomic regions located on four different linkage groups were identified to be associated with the selected traits. Positive alleles for the individual traits were contributed by both parents. A major quantitative trait locus (QTL) for high percentage total germination located on linkage group 8/18 appeared in both control and deteriorated seeds and was contributed by 'Hicks'. In contrast, 'Florida 301' donated a favorable allele for germination speed on linkage group 7 after CD only. Interestingly, the position of this locus compared well with a QTL detected in the same population, in a former study examining resistance against the black shank disease caused by *Phytophthora nicotianae*. The effects of environmental growing conditions of the mother plants on seed longevity will be discussed.

In vitro induction of tetraploid hairy roots in purple coneflower (*Echinacea purpurea* (L.) Moench)

Mehdi Alipour [a], **Ahmad Moieni[a]** and Ghasem Karimzadeh [a]

[a] *Plant Breeding and Biotechnology Department, Tarbiat Modares University, Iran*

moieni_a@modares.ac.ir

Echinacea purpurea (L.) Moench, known as purple coneflower, is a perennial herbaceous plant of Asteraceae family. It is one of the most important medicinal plant species. Some important secondary metabolites of pharmaceutical interest are accumulated in *in vitro* hairy roots. These roots can now be produced in several medicinal plants and considered as the important sources for natural secondary metabolites production. Polyploidy induction is a suitable method for increasing valuable secondary metabolites in plants. To induce the tetraploid hairy roots of purple coneflower with improved medicinal qualities, the *in vitro* diploid hairy roots were treated with 0.5, 1, 2.5 and 5 g l^{-1} colchicine for 12, 24 and 36 h. The ploidy levels of treated hairy roots were determined by flow cytometric analysis. The results showed that tetraploid induction has occurred in all tested colchicine concentrations but the use of 1 and 2.5 g l^{-1} colchicine for 36 h showed the highest tetraploidy induction. HPLC analysis also confirmed that the colchicine-induced tetraploid hairy roots had more cichoric acid (one of the caffeic acid derivatives) than diploids. Biomass and the morphology of hairy roots were also affected by tetraploidy induction.

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Development of a novel phenotyping method to assess downy mildew symptoms on grapevine inflorescences

Daniele Buonassisi[a,b], Luca Cappellin[a], Chiara Dolzani[a], Riccardo Velasco[a], Elisa Peressotti[a]* and **Silvia Vezzulli**[a]*

[a] *Research and Innovation Centre, Fondazione Edmund Mach, Italy*
[b] *Department of Agricultural and Environmental Sciences, Università degli Studi di Udine, Italy*
* *contributed equally to the study*

silvia.vezzulli@fmach.it

Grapevine downy mildew (GDM), caused by the oomycete *Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni, is one of the most important plagues affecting viticulture, especially in temperate rainy climates. *P. viticola* reduces fruit quality and yield, either by direct infection of berries or as a result of the reductions in photosynthesis and plant vigor caused by leaf infections. GDM control is based on the repeated and massive use of fungicides, leading to problems such as environmental pollution, development of resistance and residual toxicity.
The use of varieties showing durable resistance to GDM is an alternative and promising strategy to control the disease. Nevertheless, most of the *in vitro* tests developed so far for GDM resistance are focused on leaf disk bioassays that not always allow for a proper evaluation and prediction of the disease impact on grapevine inflorescence/bunch and therefore on final yield and wine quality.
In this work, we first improved the annotation procedure of foliar resistance/susceptibility under controlled conditions (optimized OIV descriptor 452-1). Second, we developed a new *in vitro* phenotyping method - from infection to symptom evaluation - for GDM resistance assessment on grapevine inflorescence, identifying the most responsive phenological stage (developed and proposed OIV descriptor 453-1). Thus, at this stage we screened several genotypes, in parallel with the optimized leaf disk bioassay, to compare the different pathogen responses between leaf and inflorescence collected from plants in untreated field. Finally, we validated our results performing the same GDM resistance assessment also on organs detached from fruiting cuttings grown in phytotrone.

Gene action of water use efficiency in coriander under well water and water stressed conditions

Hamid Dehghani[a], Mostafa Khodadadi[a], Mokhtar Jalali-Javaran[a], Sajad Rashidi-Monfared[a] and Jack T. Christopher[b]

[a] *Plant Breeding and Biotechnology Department, Tarbiat Modares University, Iran*
[b] *Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland, Australia*

dehghanr@modares.ac.ir

Enhancing effective water use of coriander (*Coriandrum sativum* L.) is a major focus for breeding to cope with drought stress, which is expressed as high transpiration efficiency (TE). Transpiration efficiency is a genetic component of water use efficiency and depends on both genetic and environmental factors. Fruit yield under water stress (WS) may be improved by identifying genetically controlled mechanisms of TE in order to identify appropriate parents for breeding program. To reach this aim, 15 half-diallel hybrids and their six parents, selected for their different responses to WS, were evaluated under well water (WW) and WS in both glasshouse lysimetric and field cultivation systems. The ANOVA on TE showed a highly significant genotypic effect for TE. General combining ability (GCA) / specific combining ability (SCA) ratio and narrow sense heritability for TE were 0.05 and 0.02, respectively while broad sense heritability for this trait was 0.77. Significant GCA and SCA effects of TE and non-significant GCA × water treatment (WT) and SCA × WT indicated that both additive and dominance gene actions are effective in expression of TE determinant traits and different water regimes did not have a significant effect on them. The GCA estimates showed that P_2 (TN-59-158) had highest value for TE while P_1 (TN-59-230) had the largest negative GCA. Positive genotypic correlation coefficient between fruit weight and TE in both glasshouse and field conditions under WW and negative genotypic correlation coefficient under WS suggest that to improve fruit yield under drought stress genotypes with lower TE must be considered which have effective water use by transporting more assimilate to fruits than other parts. Therefore, P_1 is suggested as donor in coriander improving programs for drought tolerance.

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Histochemical approaches to relation between pollen wall and fertility in natural diploid and triploid *Lilium lancifolium*

Gyung Ran Do[1] , Yoon Im Kang[1] , Jeom Hwa Han[1], Hyun Hee Han[1], Su Hyun Ryu[1], Han Chan Lee, In Myung Choi[1] and Ju Hee Rhee[2]

[1]*National Institute of Horticultural & Herbal Science, RDA, Suwon, Rep. of Korea*
[2] *National Agrobiodiverdity Center, National Academy of Agricultural Science, RDA, Jeonju, Rep. of Korea*

rheehk@korea.kr

Lilium lancifolium is the only natural polyploidy-complex species involving both diploid and triploid plants in the genus *Lilium*. Generally it is reported that triploid plants are self-sterile while diploid plants are self-fertile and most of the research has been limited mainly to chromosomal studies that overlooked histological. We studied the correlations between pollen fertility and pollen wall structures by comparing diploid and triploid pollen grains with histochemical methods. In this comparative investigation, we used various microscopy techniques including histochemistry. The significant structural features of triploid pollen grains differentiated from diploid pollen grains revealed an abnormalities of pollen surface layers, exine and intine in maturing pollen grains, which were excessive reserves of lipids including pollenkitt accumulated on the exine and erratic development of the intine layers and finally resulted in pollen grain unfolding and male-sterility. From observing the series of histochemical events that induced male-sterile pathway in natural triploid pollens, the present study showed a speciality in natural male-sterile pollens besides correlations between pollen wall structures and pollen fertility. Our results propose the necessity and importance of further research on natural polyploid-ontogenetic diversity.

Medicinal plant with potential antimicrobial activity harbor a high presence of antagonistic rhizobacteria

Dilfuza Egamberdieva[a,b], Stephan Wirth[a], Undine Behrendt[a] and Gabriele Berg[c]

[a]*Institute for Landscape Biogeochemistry, Leibniz Centre for Agricultural Landscape Research (ZALF), Müncheberg, Germany*
[b]*National University of Uzbekistan, Tashkent, Uzbekistan*
[c]*Institute of Environmental Biotechnology, Graz University of Technology, Graz, Austria*

dilfuza.egamberdieva@zalf.de

The composition of biologically active compounds of medicinal plants varies widely depending on the plant species, climatic condition, and soil type and on their association with microbes Although a vast number of medicinal plants have been well studied with respect to their phytochemical constitutes and pharmacological properties, their microbiome and the physiological interactions between host and microbes remain poorly understood. Therefore, the current exploratory study was designed to evaluate whether medicinal plants, *Zizifora capitata* L. (Field basil) and *Hypericum perforatum* L. (St John's wort) from the Chatkal Biosphere Reserve of Uzbekistan with contrasting antimicrobial activities have an impact on the diversity of root-associated cultivable endophytic bacteria and their physiological properties. *H. perforatum* was proved to possess potential antimicrobial activity against a wide range of pathogenic bacteria and fungi, whereas the extract of *Z. capitata* did not exhibit any inhibitory activity against the tested microbes. Our data reveal that host plants that differ in their antibacterial activity exhibited consistently selective effects on endophyte diversity and colonization. The endophytes associated with *H. perforatum* were more diverse (*Arthrobacter, Bacillus, Erwinia, Pantoea, Serratia* and *Stenatrophomonas*) than the endophytic isolates from *Z. capitata* (*Achromobacter, Bacillus, Enterobacter, Pantoea and Pseudomonas*). We have observed that endophytic bacteria associated with both investigated plants exhibited multiple plant beneficial activities, such as the production of IAA, HCN and cell-wall-degrading enzymes. In addition to the higher diversity of endophytes associated with *H. perforatum*, this work documents evidence for an increased antagonistic activity compared with endophytes of *Z. capitata*. Interestingly, almost half of the endophytes associated with *H. perforatum* exhibited an inhibitory effect on plant growth, whereas a plant inhibitory effect was not observed among the isolates from *Z. capitata*. The plant extract of *H. perforatum* contains more phenolic compounds that may play a role in the inhibition of plant growth. The endophytic isolates, which exhibited antagonistic activity against a wide range of fungal pathogens and stimulated plant growth, exhibited statistically significant reduction of tomato root rot compared with the *Fusarium*-infected control tomato plants. Our results provide important knowledge on the diversity of cultivable endophytic bacteria associated with the medicinal plants *H. perforatum* and *Z. capitata* and contribute to a better understanding of endophytes that possess a number of traits that are beneficial for plant growth and development. The phytochemical constituents of medicinal plants play an important role in the selection of their endophytes. Our study highlights the fact that the physiological properties of endophytic bacteria are closely linked to the biological properties of their host, as plants with antibacterial activity support microbes with strong antagonistic activity. The antagonistic strains were able to control tomato root rot caused by *F. oxysporum* and stimulate plant growth under greenhouse conditions. These results suggest that endophytic bacteria associated with certain medicinal plants could be a cost effective source for agro-based biological control agents and may successfully be used to promote plant growth and protect plants from fungal disease.

Response mechanisms of hop (*Humulus lupulus* L.) plant under drought stress

Zala Kolenc[1], Dominik Vodnik[2], Stanislav Mandelc[2], Branka Javornik[2] and **Andreja Čerenak[1]**

[1] *Slovenian Institute of Hop Research and Brewing, Slovenia*
[2] *Biotechnical Faculty, University of Ljubljana, Slovenia*

andreja.cerenak@ihps.si

Plants are exposed to several abiotic stresses, all of which affect growth and development, with an impact on crop quality and quantitiy. Drought, causing a water deficit, is one of the most important constraints to crops, including hop (*Humulus lupulus* L.). We studied the hop response to drought stress by combining two approaches: proteomics and physiological measurements. Two cultivars, Savinjski golding and Aurora were exposed to progressive drought in a pot experiment and sampled/measured at different drought stages (mild, moderate, severe). Water deficit induced changes in chlorophyl fluorescence and gas exchange parameters (net photosynthesis, stomatal conductance, transpiration) were evaluated with respect to soil water content and relative water content in leaves. 2D-DIGE proteomic analysis was used to compare differences in protein abundance between control and stressed plants. Furthermore, based on statistics, 44 differential spots were subjected to mass spectrometry. For all 44 spots, two-way ANOVA showed a statistically significant difference with respect to time and/or drought conditions. We identified 28 proteins differentially expressed in drought-stressed plants by using tandem mass spectrometry (MALDI-TOF/TOF). They are involved in photosynthesis, energy and nitrogen metabolism, glycolysis, Calvin cycle, the oxidation-reduction process and reactive oxygen species pathway. In addition, this study revealed some proteins, such as glutamine synthetase, auxin-binding protein and lactoylglutathione lyase, which contribute to our understanding of hop response mechanisms under drought stress. Rather surprisingly, this very first proteomic analysis of drought stressed hop showed no other differentially expressed proteins that are typically reported for this type of stress. Further analyses, which could include targeted leaf sampling, more thorough identification of proteins and analysis of metabolites, should give us an accurate insight into hop response to water deficit.

Genetic resources of cultivated forms of beet (*Beta vulgaris* L.) as potential donors for breeding

Kamilla Kuzdowicz

Plant Breeding and Acclimatization Institute-National Research Institute, Poland

k.kuzdowicz@ihar.bydgoszcz.pl

Genetic variability of the plant material is necessary for breeding new cultivars. Sugar beet has a relatively narrow genetic ground and new sources for breeding programs are needed. If in the breeding programmes for monogerm seeds and another agronomic important traits only hybridization methods which based only on Owen-type cytoplasmic male sterility (CMS) will be used, genetic erosion within this species will be caused. Similarly in fodder beet the utilisation of CMS source derived from sugar beet caused the narrowing of the root shape and dry matter content. Because of this agronomic value of the beet cultivated forms collected in National Centre for Plant Genetic Resources in Poland was described. Investigated accessions were originated from internationals expeditions (local population mainly) and national breeding station.

Two field experiments were conducted in Central Poland under randomized split-block design with two or four replication. As a control cultivars with good agronomic value were used. After harvesting fresh roots were processed using standard Venema automatic beet laboratory system. The most important traits such as plant morphology (weight of leaves, weight, size, shape and skin colour of root, depth in soil, root and dry matter yield) and chemical composition of roots (sugar content, potassium, sodium and α-amino nitrogen) were described. A great variability both between and within population, especially in fodder beet accessions, was found and they can be used as a sources for breeding programmes.

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Genetic diversity and analysis of antimicrobial properties of selected oregano clones for use in the aquaculture industry

Charikleia I. Papaioannou[a,b], Michalis K. Stefanakis[c], Elias Anastasopoulos[d], Pavlos Makridis[b,e], George Tsikalas[c], Dimitris Zaragotas[d], Haralambos E. Katerinopoulos[c] and **Vassilis Papasotiropoulos[a]**

[a] *Department of Agricultural Technology, Technological Educational Institute of Western Greece, Greece*
[b] *Department of Biology, University of Patras, Greece*
[c] *Department of Chemistry, University of Crete, Greece*
[d] *Department of Agricultural Technology, Technological Educational Institute of Thessaly, Greece*
[e] *Institute of Aquaculture, Hellenic Centre for Marine Research, Greece*

vpapasot@teimes.gr

According to the EU directive (European Parliament and Council Regulation (EC) No. 1831/2006), the use of antibiotics in the aquaculture industry has been restricted. Oregano oil is a natural product, well known for its antibacterial properties and a possible candidate for the replacement of antibiotics. In this study, thousands of oregano seedlings were screened in order to identify freezing resistant ones, assuming that as long as resistance increases, the content of secondary metabolites would also increase. Oil from the selected oregano clones was extracted and the total phenolic content was determined. GC–MS analysis was also applied in order to determine the chemical composition of oregano oil samples. *Vibrio* bacteria growing in solid media and in rotifer cultures were used to test *in vitro* the antibacterial properties of the extracted oil. Apart from that, genetic screening using molecular markers was also applied for the identification of the selected oregano plants, for securing their legal protection, and also to identify candidate markers that can be utilized in plant breeding. The selected clones were screened using twelve microsatellite markers (SSRs). According to our data, a) a significant amount of genetic diversity (Nei's, 1972 genetic distance values ranged from 0 to 1,79) has been revealed among the clones under study, b) the SSR loci examined exhibited a high degree of genetic polymorphism revealing several alleles and c) several specific loci with discrete fingerprinting pattern can be used as diagnostics to identify and discriminate the different plants. Moreover, a strong correlation between the genetic structure and the chemical composition of the selected clones has also been found. We plan to further explore the potential of these oils for the disinfection of endo- and ectoparasites of various aquatic organisms, and also to apply those markers in plant breeding schemes.

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Characterising cannabinoid composition in diverse *Cannabis* germplasm to accelerate the genetic metabolic engineering of chemotype-specific cultivars

Matthew Welling[a,b], Lei Liu[a], Tim Shapter[a,b], Carolyn A. Raymond[a] and Graham J. King[a]

[a]*Southern Cross Plant Science, Southern Cross University, Australia*
[b]*Ecofibre Industries Operations, Australia.*

matt.welling@scu.edu.au

The accurate characterisation of chemical phenotype or chemotype is important for the development of *Cannabis* cultivars which are utilised in the production of cannabinoid-based botanical drug products. Despite the availability of several methods for chemotyping and genotyping as a means of qualifying and quantifying cannabinoid composition, only a fraction of the gene pool has been examined. Using a combination of liquid chromatography–mass spectrometry (LC–MS) cannabinoid profiling as well as dominant and co-dominant DNA sequence characterised amplified region (SCAR) markers, we conducted a representative survey of a broad range of diverse *Cannabis* subtaxa, including European and Asian fibre accessions and eight drug accessions of mixed origin. Cannabinoid compositional variability across the gene pool was significantly greater in heterozygote genotypes than has previously been recorded. These results suggest that in order to identify explicit chemotypic variants for the pre-breeding of pharmacological-specific cultivars, a comprehensive surveying strategy, which considers the co-dominant SCAR marker, as well as exhaustive cannabinoid profiling, is required. Characterisation of the genomic determinants underlying chemotype using high throughput DNA sequencing may improve the accuracy of predicting cannabinoid composition and promote the genetic and metabolic engineering of *Cannabis* for pharmacological development.

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Chrysanthemum transcriptome analysis by 3rd generation sequencing

So Youn Won[a], Jae-A Jung[b], Jung Sun Kim[a], Sangho Kang[a], Beom-Seok Park[a] and Seong-Han Sohn[a]

[a] *National Academy of Agricultural Science, RDA, Republic of Korea*
[b] *National Institute of Horticultural and Herbal Science, RDA, Republic of Korea*

soyounwon@korea.kr

Obtaining complete gene set with full-length coverage is important for many studies in biology. Although whole genome sequence and underlying gene models were uncovered for many species and the current DNA sequencing technology would accelerate the accumulation of genomic information, completion of genome sequencing takes a lot of effort. Chrysanthemum is still a recalcitrant species due to polyploidy, repetitive sequence and huge genome size. For species without genomic sequences, transcriptome has been actively analyzed in various conditions such as developmental stage, biotic or abiotic stress and hormone treatment etc. Since reads generated by the most popular 2nd generation sequencer are usually shorter than the actual transcripts, sequencing reads are definitely assembled into longer one and consequently assembled gene set might be incomplete in terms of contiguity. However, the 3rd generation sequencer, i.e. PacBio's single-molecule long-read sequencer is known to generate longer sequence with an average of 10-15kb in length and be effective to recover transcripts in full-length. Although transcriptome in *Chrysanthemum* species were already analyzed using next generation sequencer by other researchers, we decided to improve the quality of gene set by using PacBio's platform. Total RNA in *C. morifolium* was extracted and subjected to library construction. During library preparation, cDNA was size-fractionated into three levels (1-2kb, 2-3kb and 3-6kb) in order to prevent the preferential sequencing of short transcripts and conversely enrich the information on longer transcripts. Generated library was sequenced by PacBio's RS II with P6-C4 chemistry. Raw reads were processed and clustered into high-quality of isoforms representing transcripts by ICE and Quiver program. Error-prone long reads were compensated by correcting errors with high-quality of reads generated by Illumina's HiSeq2000 by LSC program. The function of gene set was annotated by BLASTX and Viridiplantae protein database. We report the overall procedure for transcriptome sequencing using the new sequencer and data analysis and provide the detailed information on chrysanthemum transcriptome.

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Chrysanthemum genome project

So Youn Won[a], Seulki Lee[a], Jae-A Jung[b], Jung Sun Kim[a], Sangho Kang[a] and Seong-Han Sohn[a]

[a] *National Academy of Agricultural Science, RDA, Republic of Korea*
[b] *National Institute of Horticultural and Herbal Science, RDA, Republic of Korea*

soyounwon@korea.kr

Today, for about a hundred of plant species the genome has been sequenced and the progress in sequencing technology and bioinformatics is expected to further boost *de novo* genome sequencing for other species in plant kingdom. Genomic information serves as a fundamental resource in basic and applied researches, and particularly its incorporation to the phenotypic data at the population level offers a new paradigm in plant breeding programs. After the first release of the genome sequence on the model plant, *Arabidopsis*, plants for food or feed like cereals, vegetables and fruits have been the major target for genome project, whereas floricultural species are rarely sequenced. Chrysanthemum is an important floricultural species and belongs to Anthemideae tribe in Asteraceae/Compositae, the largest family of angiosperms together with Orchidaceae. Asteraceae includes the economically valuable species such as sunflower, lettuce and yacon as well as agronomical important weeds. Itis distinct in its inflorescence head structure, pollen presentation and contains plenty of secondary metabolites. Although several genomic studies on chrysanthemum have focused to profile the transcriptome in the aspect of the flower development and some characteristics critical for cultivation such as disease, noxious insect, cold and dehydration stress, the lack of complete genomic information would drag the in-depth study on chrysanthemum. Therfor, we started the genome sequencing on *Chrysanthemum* species. Although the technique for sequencing and computation has been unprecedentedly advanced, the hexaploid and gigantic genome of cultivated *C. morifolium* is probably difficult to be completely sequenced. To overcome this, diverse *Chrysanthemum* species were collected in *Korean* peninsula and a diploid wild species (*C. boreale*) was chosen for the *Chrysanthemum* genome project. Genomic DNA was extracted, subjected to library construction and sequenced using Illumina's HiSeq platform. After raw reads were quality-checked, preprocessed and error-corrected, reads with high quality were used for the downstream analysis and assembly. K-mer frequency analysis suggests that the genome is highly heterozygous, repetitive and around 3 Gb in length. Using SOAPdenovo2 and SSPACE programs, short reads were assembled into contigs and scaffolds. To improve the quality of genome assembly in terms of coverage and contiguity, Illumina's TruSeq Synthetic Long Reads strategy similar to traditional BAC-pooling sequencing was also utilized. Here, we report the detailed information on chrysanthemum genome sequencing.

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Cryopreservation of pear germplasm by encapsulation-dehydration

JungYoon Yi[a], GiAn Lee[a], YoungYi Lee[a], GwagJae Gyun[a], EunHo Son[a] and HongJae Park[a]

[a] *National Institute of Agricultural Sciences, RDA, Republic of Korea*

naaeskr@korea.kr

Pear (*Pyrus* species) is an important horticultural germplasm grown widely in South Korea. The preservation of pear germplasm is troublesome like other clonal germplasm, since whole plants or their tissues need to be conserved. Among the currently available methods for long-term conservation of clonal germplasm, cryopreservation of shoot tips is the most reliable, cost- and space-effective option. In this study, alginate-coated axillary shoot tips from *in vitro* grown pear were successfully conserved in liquid nitrogen following dehydration. *In vitro* shoot tips were precultured in MS medium with increasing sucrose (0.3 and 0.5M) for 24 hours and 16 hours, respectively. The precultured shoot tips were coated with sodium alginate containing 0.5M sucrose and treated in a medium supplemented with 0.8M sucrose for 16 hours at 25℃. Beads containing one shoot tip were then dehydrated up to about 28% water content (fresh weight basis) on sterile dry silica gel at 25℃ and immersed in liquid nitrogen. The average rate of shoot formation for 'BaeYun No.3' and 'Whanggeum' after warming was about 55.7% and 43.3%, respectively. This method appears to be a promising technique for cryopreserving shoot tips from in-vitro grown plantlets of pear germplasm.

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Parallel flash and poster presentations: Vegetables

Who is sowing our seeds? The role of the UK Vegetable Genebank in supporting plant breeding and research

Laura Davies, and **Charlotte J. Allender**

School of Life Sciences, University of Warwick

charlotte.allender@warwick.ac.uk

The UK Vegetable Genebank was opened in 1980 with a remit for the conservation and management of crop genepool diversity. The UKVGB currently holds approximately 14,000 seed samples which represent commercial varieties, landraces and crop wild relatives. The collections focus on vegetable crops, particularly *Brassica*, *Lactuca* (lettuce), *Daucus* (carrot) and *Allium* (onion and related crops), with smaller collections of other vegetables such as radish, celery, parsnip and leafy salad crops. Seed is available on request for use in plant breeding and research, but historically not all users have reported published findings back to the UKVGB. Understanding how genebank material is used is valuable for management purposes and for enabling future users to be aware of previous work associated with individual accessions. A systematic review of published studies which utilized UKVGB material was carried out in order to collate the articles and understand the range of topics researched using the material. A total of 271 publications were identified, with research topics ranging from pathology through to biogeography and evolution. The number of studies published per year shows a significant increase between 1980 and 2015. More recent efforts to increase the accessibility of UKVGB material through the construction of core collections and diversity sets is also reflected in the publications identified. The overall results indicate that the UKVGB collections have provided genetic resources which have underpinned a significant amount of research both within the UK and elsewhere in the world, highlighting the value of such collections for research as well as breeding. The collation of published data will provide a useful tool to assist collection managers and potential users in understanding and selecting appropriate material for future work.

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Whole genome resequencing in *Cynara cardunculus*: detection of intra-specific variability and the identification/annotation of novel polymorphisms

Alberto Acquadro[a], Lorenzo Barchi[a], Ezio Portis[a], Giulio Mangino[a], Danila Valentino[a], Giovanni Mauromicale[b], **Sergio Lanteri[a]**

[a] *DISAFA, Plant Genetics and breeding, University of Torino, Italy*
[b]*Di3A, University of Catania, Italy*

sergio.lanteri@unito.it

Cynara cardunculus L. (2n=2x=34, Compositae a.k.a. Asteraceae family) is native to the Mediterranean basin and includes the botanical taxa globe artichoke (var. *scolymus*), cultivated cardoon (var. *altilis*) and their ancestor wild cardoon (var. *sylvestris*). The primary product of globe artichoke is the immature inflorescence (head or capitulum), while cultivated cardoon is exploited for the production of fleshy stems.

Italy is the top producing country of globe artichoke and harbors the richest primary cultivated gene-pool which, on the basis on capitulum traits, is classified in: i) 'Spinosi' with spines on heads/leaves; ii) 'Violetti', with medium-sized, violet-colored heads; iii) 'Romaneschi', with spherical or sub-spherical heads and iv) 'Catanesi', with relatively small and elongated heads.

We recently released the first reference genome sequence of globe artichoke (www.artichokegenome.unito.it). Here we report on the polymorphisms obtained following the comparison of resequencing data (Illumina, 2PE x 150 bp; coverage: 40X) of four globe artichoke (representative of the 4 main varietal types) and one cultivated cardoon genotypes with the reference genome (Bwa/Samtools-based pipeline). By combining iterative mapping and reference guided-assembly (IMR-Denom, http://mus.well.ox.ac.uk/) the five genomes were reconstructed and annotated using the Maker-P suite (http://www.yandell-lab.org).

A total of ~61M SNP/indel (~38M in heterozygous and ~23M in homozygous form) were discovered. The SNP variation ranged from 6,34M in cultivated cardoon to ~14,50M in the Violetto genotype. A snpEff (http://snpeff.sourceforge.net/) analysis on functional SNPs revealed that ~57% of homozygous SNPs were located in intergenic regions, ~8.7% in intronic region, and ~1,4% in coding sequences. The Spinoso, Violetto, Catanese and Romanesco genotypes showed 113k, 96k, 109k, 89k of coding homozygous SNPs respectively, while 47k were detected in cultivated cardoon. In coding sequences, the 53.4% of variations led to synonimous mutations, 46% to non-synonymous amino acid changes and 0.6% to non-sense mutations. Some missense SNPs, present in genes involved in the biosynthesis of secondary metabolites (caffeoylquinic acids and sesquiterpene lactones) and seed yield related genes, were explored using Provean (http://provean.jcvi.org), and the qualitative impact of functional variants highlighted.

A total of 321 PAV (Presence/Absence Variation) were identified among the four globe artichoke genomes in respect to reference one: 190 genes (59%) were absent in the 4, 70 (22%) in one, and 45 (14%) in 2 varietal types. Interestingly, 16 genes (5%) were genotype-specific: functional annotation will be used to inspect their role in phenotypic variations.

This study represents the first whole genome resequencing experiment in *Cynara cardunculus*. Substantial genetic differences between the five accessions in study was found. The emerging DNA markers represent key tools for genetic traceability of semi-processed or processed globe artichoke italian germplasm along the food chains, thus supporting the creation of quality labels.

Investigation for suitability of three new interspecific rootstocks for eggplant grafting aiming to breeding purposes

Krommydas S. Konstantinos[a], **Athanasios G. Mavromatis[a]**, Fotios A. Bletsos[b] and Demetrios G. Roupakias[a]

[a] *Laboratory of Plant Breeding, Department of Field Crops and Ecology, School of Agriculture, Faculty of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, Greece*

[b] *Department of Horticulture, Hellenic Agricultural Organization "Demeter", Greece*

amavromat@auth.gr

The utilization of CMS eggplant lines in interspecific crosses with related species may facilitate the production of useful genetic material and interspecific hybrids with potential for eggplant rootstocks. In a previous study, we developed the CMS lines of the commercial Greek eggplant cultivars 'Langada', 'Emi' and 'Tsakoniki' (cmsL, cmsE and cmsT, respectively). These lines were crossed with *S. integrifolium* (SI) and *S. gilo* (SG) which are resistant to *Fusarium* wilt and as a consequence five interspecific hybrids viz. F_1(cmsLxSI), F_1(cmsExSI), F_1(cmsTxSI), F_1(cmsExSG) and F_1(cmsLxSG), were produced. In this experiment, the suitability of these interspecific hybrids as rootstocks for eggplant grafting, was investigated by using the cleft grafting technique and the aforementioned eggplant cultivars as scions. For this purpose, the percentage of successful graft combinations, the parameters of early and total fruit number, fruit weight and fruit morphology, were recorded. Fifteen scion/rootstock combinations were used in comparison to three self-grafted applications on to commercial eggplant cultivars, using as controls. According to the results, the successful grafting percentage in all combinations was high and comparable to the self-grafted controls, indicating high scion/rootstock compatibility. In general, grafting on the interspecific rootstocks did not significantly affect the early production in eggplant. However, grafting on F_1(cmsTxSI) and F_1(cmsExSG) had an overall positive effect on the total production of all three eggplant cultivars, whereas the effect of F_1(cmsLxSI) was positive only in combination with cv. 'Langada'. In addition, grafting on some interspecific hybrids resulted in alterations on the morphology of the eggplant fruit (shape & color) which varied depending on the scion/rootstock combination. The results of our research indicated that interspecific hybrids F_1(cmsTxSI) and F_1(cmsExSG) have high potential as rootstocks for eggplant grafting and may be considered useful for further evaluation. Furthermore, in our studies we will organize an action to investigate the effect of these rootstocks on the fruit quality parameters and sensory properties in eggplant cultivars aiming to the satisfaction of the consumer perspectives.

Development of a new screening method for resistance towards *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) in tomato

Mas Muniroh Mohd Nadzir[a], Sjaak van Heusden[a], Flavia Vieira Lelis[b] and Jan van der Wolf[b]

[a] *Plant Breeding, Wageningen UR, The Netherlands*
[b] *Bio-interactions and Plant Health, Plant Research International, The Netherlands*

mas.muniroh@wur.nl

The tomato pathogen, *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), is a quarantine organism in Europe and many other parts of the world. It is considered as the most severe bacterial pathogen affecting tomato. Infection can occur through infected seeds, or mechanically from plant to plant, or from contaminated soil. One of the most effective ways to prevent an outbreak of the pathogen is through resistance breeding. There are several wild tomato species that are tolerant against Cmm. *Solanum arcanum* LA2157 is known to have high levels of Cmm resistance and was used as donor in our breeding program. We developed Near Isogenic Lines (NILs) and combiNILs in a *S. lycopersicum* cv Moneymaker background. Our current interest is to fine map the known QTL regions located on chromosome 7 and 9. To fine map the QTL regions we plan to do a recombinant screening. A challenge that we are facing is the limited space and strict regulations for screening with the quarantine pathogen. In order to facilitate fine mapping, we are developing a new method to inoculate. *In vitro* plants will be inoculated using different methods, scoring will be done based on the wilting symptoms, a TaqMan assay and if necessary selective plating for quantification. The best procedure of inoculating the plants axenically will then be used in our large scale recombinant screening project.

Changes in carotenoid metabolism in response to biotic and abiotic stresses in various carrot genotypes

Florent Perrin[abc], Séverine Gagné[a], Dorine Laloi[c], Tricy Oublié[c], Cécile Dubois-Laurent[b], Sébastien Huet[b], Didier Peltier[a], and Emmanuel Geoffriau[b]

[a] *IRHS, SFR 4207 QUASAV, Université d'Angers, France*
[b] *IRHS, SFR 4207 QUASAV, Agrocampus Ouest, France*
[c] *IRHS, SFR 4207 QUASAV, INRA, France*

florent.perrin@univ-angers.fr

Carotenoids represent an important class of human health metabolites as precursors of vitamin A. The carrot root, a worldwide consumed vegetable contains high carotenoid content. Selection events led to diverse types of root color related to specific carotenoid accumulation (Clotault et al., 2012; Soufflet-Freslon et al., 2013): α-carotene and β-carotene in orange genotypes, lutein in yellow genotypes, lycopene in red genotypes and no carotenoid in white genotypes (Clotault et al., 2008; Surles et al., 2004). Carrot represents an interesting model to understand the accumulation in these pigments.

If some knowledge about genetic determinism exists (Arango et al., 2014; Jourdan et al., 2015), a few studies have highlighted the impact of environmental factors on the accumulation of carotenoids in carrots (Perrin et al., 2016). This work thus aims to (i) determine the impact of individual and combined stress (water restriction and *Alternaria dauci* inoculation) on carotenoid accumulation in carrot genotypes, and (ii) determine if carotenoid content variations could be explained by carotenogenesis gene expression.

Stresses were applied separately or in combination on a panel of six genotypes with various root colors (orange, purple, red and white). Our results showed that individual stresses impact carotenoid accumulation but the effect depends on the genotype. Combined biotic and abiotic stresses condition led to reduced carotenoid contents for all genotypes which can be related to whole plant metabolism. This study provides new knowledge on genetic control of carotenoid accumulation and leads to perspectives about breeding for product quality in carrot and vegetable crops.

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Identification of quantitative trait loci (QTL) linked to increased lateral root emergence and growth in an intra-specific *Lactuca sativa* cross for the improvement of lettuce transplants.

Justin N. Roberts[a], James Monaghan[a], David Pink[a], Martin Broadley[b] and Paul Hand[a]

[a]*Fresh Produce Research Centre, Crop and Environment Department, Harper Adams University, UK.*
[b]*Plant and Crop Sciences Division, School of Biosciences, University of Nottingham, UK.*

jnroberts@harper-adams.ac.uk

In Western Europe lettuce *(Lactuca sativa)* predominantly planted in the field as transplants at the 4-5 leaf stage to increase crop uniformity and yield by reducing abiotic stresses that may influence germination time and seedling growth, such as thermoinhibition and weed infestation. Lettuce transplants often have a modified root system compared to directly drilled lettuce with the tap root being mechanically or air pruned due to the constraints of the small transplant block, leaving the crop with a minimalised tap root from which primary lateral roots are able to emerge.

A root system needs to develop within the days following planting to maintain plant growth. Compared to the tap root, lateral roots are commonly found in upper soil layers which can exacerbate the effects of mild drought when water may only be available deeper in the soil profile and lateral root growth is known to cease. Hence, developing a larger root system, with deeper rooting through rapid lateral root development may be a desirable trait in cultivated lettuce transplants could increase crop uniformity and yield while having the additional benefit of being able to reduce irrigation and fertilizer use.

This study screened seedlings of the *L. sativa* intra-specific Saladin X Iceberg mapping population in a high throughput assay and identified genetic variation for lateral root growth and emergence. Lines that displayed a rapid lateral rooting phenotype in seedlings also showed a reduced establishment time following root pruning and transplanting. QTL and DNA markers linked with primary lateral root emergence and lateral root growth were identified and will allow marker- assisted selection in the development of cultivated lettuce lines, specifically for transplant production, with the ability to establish more quickly in the field.

Deciphering diversity in the origins of pepper (*Capsicum* spp)

Cristina Silvar[a] and Carlos A. García-González[a,b]

[a] *Departamento de Bioloxía Animal, Bioloxía Vexetal e Ecoloxía, Universidade da Coruña, Spain*
[b] *Unidad Acádemica de Ciencias Químicas y de la Salud, Universidad Técnica de Machala, Ecuador*

c.silvar@udc.es

Pepper (*Capsicum* spp) is one of the most important vegetables and spice in the worldwide trade, because of its versatility for culinary and pharmaceutical usages. The genus *Capsicum* belongs to the Solanaceae family and harbours thirty-eight species, of which five are domesticated: *C. annuum* L., *C. chinense* Jacq., *C. frutescens* L., *C. baccatum* L. and *C. pubescens* Ruiz et Pav. In the last decades several attempts have been devoted to investigate the genetic and phenotypic diversity harboured by this genus, particular efforts being directed to old varieties or landraces. However, further investigations aimed at accurately defining the relationships among species as well as identifying geographic regions harboring the higher variability are required.

In the present work a collection of 107 pepper landraces, representative of the five domesticated species from the Andean region (Ecuador, Peru and Bolivia), which is the *Capsicum* spp primary center of diversification, was assessed with a set of sixteen microsatellite markers. In total, 187 alleles were detected in the Andean collection of which 49.7 % were unique alleles. Genetic diversity, expressed as a measure of the Nei´s unbiased gene diversity index was very similar (around 0.79) in the three countries. The highest diversity was found in Ecuador for *C. frutescens*, in Peru for *C. chinense* and in Bolivia for *C. annuum*.

The genetic diversity of Ecuadorian, Peruvian and Bolivian peppers was compared to the one of a panel of 65 worldwide *Capsicum* landraces, mostly originating in the Mediterranean region, one of the secondary centers of crop domestication. A factorial correspondence analysis performed independently on each domesticated species revealed geographic patterns of genetic differentiation. The presence of well-defined gene pools according to the origin suggests that different geographic groups underwent an independent evolutionary history, driven by climatic adaptations and human selections. This was supported by the presence of several exclusive alleles not only in Ecuador, Peru and Bolivia but also in the non-Andean countries and some of which are already fixed. *Capsicum* accessions from the Andean countries were found less differentiated in respect to those of non-Andean regions. This might reflect a more continuous gene flow between these adjacent geographical regions.

The notably degree of genetic diversity found within the landraces in study makes them suitable for the exploration of traits of interest for breeders, growers and consumers.

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Genetic inheritance resistance to Yellow Virus in melon (*Cucumis melo* L.)

Entit Hermawan [a], Darda Efendi [a] and **Ridwani Sobir** [a]

[a] *Department of Agronomy and Horticulture, Bogor Agricultural University, Indonesia*

rsobir@yahoo.com

Yellow virus (YV) diseases cause severe damage to the crops, since inhibit plant vegetative growth and made total lost production in melon. Development of resistant variety is the most effective mean to control the diseases, since the vector of white fly is polyphagia, therefore difficult to control. The research consist of two field experiments, there were evaluation of resistance to YV and parent selection, and evaluation of resistance in P1, P2, F1 and F2 population. Twenty genotypes from three major melon groups of cantaloupe, inodorous and dudaim were evaluated and one line of dudaim group (D1) exhibits high resistance to YV, however other lines belong to cantaloupe and inodorous showed highly susceptible to the virus. Resistance analysis on F1 of crossing resistance x susceptible parents resulted resistance of all F1 population, subsequently resistance evaluation in F2 population revealed the disease severity score was not following normal distribution, that indicated that resistance to YV was controlled by a major gene. Chi-square ($\chi 2$) test result revealed 13:3 as a suitable ratio indicated that a pair of 2 genes with dominant and epistasis recessive action controlled the resistance to YV. PCR analysis on infected leaves showed virus existence in resistant genotypes with lower scale compare to the susceptible genotypes indicated the genes controlling resistance to the virus not to the vector.

Lactuca aculeata and *L. georgica* are wild lettuce species, harboring unique genetic resources for crop improvement

Alex Beharav

Institute of Evolution (IOE), University of Haifa, Israel

abeharav@univ.haifa.ac.il

The genus *Lactuca* comprises about 100 wild species, mainly distributed in the Northern Hemisphere. Cultivated lettuce, *Lactuca sativa* L., is one of the most important and widely distributed leafy vegetables. Domestication has resulted in limited genetic variation in the cultivated crop making it vulnerable to diseases, pests, and environmental stresses. *L. sativa* is part of a reproductively isolated group that includes the wild *species L. serriola*, *L. saligna* and *L. virosa*. Of these three wild species, only *L. serriola* represents the primary gene pool. Six other wild species are taxonomically close to the cultivated lettuce: *L. aculeata*, *L. scarioloides*, *L. azerbaijanica*, *L. georgica*, *L. dregeana* and *L. altaica*. However, these are less widespread and the status of some of them as distinct species is unclear. Studies are ongoing to clarify their taxonomic status and crossing potential.

Current information on the genetic diversity of wild *Lactuca* germ-plasm is mostly based on gene bank accessions originating from Europe. Less attention has been paid to accessions that representing species from southwest Asia that is the center of diversity for wild lettuce species closely related to *L. sativa*. Extensive collection and studies of wild populations of *Lactuca* spp. using unique germ-plasm originating from the center of diversity have started in IOE during recent years. These studies based mainly on new collections of *L. serriola*, *L. aculeat*a, *L. saligna*, and *L. georgica* from Israel and Armenia, as well as a few accessions collected in past from Jordan, Turkey and addition countries. The objectives of the research are related to the collecting of the wild *Lactuca* species.

In this presentation I'll focus on identification and collection's strategy of unique germ-plasm as well as novelty results obtained in our studies with *L. aculeat*a and *L. georgica*, both representing the primary lettuce gene pools. Specifically, the results are devoted to: species definition; taxonomic validation; morphological traits; identification of natural putative hybrids (*L. aculeata* x *L. serriola*) ; genetic diversity; out-crossing rate; diseases resistance (*Bremia lactucae* - *L. aculeat*a); chemical composition; and bolting control (*L. georgica*). Variation obtained in our studies suggest that these two species are largely untapped sources for breeding resistances against biotic and a-biotic stresses, as well as other traits, into the cultivated lettuce. All results are saved in the gene-bank information system of the Wild Lettuce Gene Bank (WLGB) project of IOE using Microsoft Access 2013.

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Described and ongoing results have obtained by various levels of national and international collaborations that will aknowledge during the presentation.

Yield and quality of melon cultivars and their F_1 hybrids influenced by field salinity stress

Mahmoud Akrami and Ahmad Arzani

Department of Agronomy and Plant Breeding, Isfahan University of Technology, Isfahan, Iran

Mahmoud.akrami@ag.iut.ac.ir

Increasing stress tolerance is one of the most important goals of plant breeding. Salinity is a major abiotic stress limiting crop production in many agricultural regions of the world. Iran is one of the countries that most profoundly affected by salinity. In Iran, salinity levels in the soil and groundwater are a concern and in some areas this salinity has already reached a critical level for horticultural crops, especially melon. Therefore, new breeding strategies should be implemented in order to best meet the criteria of saline agriculture through development of salinity tolerant hybrids in melon. The present study was conducted to evaluate the effects of salinity on the yield and quality of melon genotypes grown in normal and salinity stress under field conditions. Ten melon genotypes including Rishbaba and Sabouni as salt-tolerant, Magasi and Gargar as salt-sensitive cultivars and their F_1 hybrids were used. Salinity was applied with saline water irrigation (EC = 14 dS/m). Yield and total soluble solids of melon fruits were measured. In addition, sugar compositions including fructose, glucose and sucrose were measured using a NH_2 column of HPLC system. Results of combined analysis of variance indicated that the genotypes highly significantly differed for the studied traits. Salinity stress also influenced significantly all the traits. The results revealed that salinity dramatically reduced fruit yield (around 45 %) while the total soluble solids and the sugar components of fruits significantly increased. Fruits yield of genotypes ranged from 1.7 to 4.7 kg and 1.2 to 2.4 kg for normal and stress conditions, respectively. Also, total soluble solids of fruits ranged from 5.9 to 7.4 % and 7.2 to 11.8 % for normal and stress conditions, respectively. The sugar compositions ranged from 13 to 20.9 and 13.2 to 26.2 mg/gFW for fructose, from 7.9 to 16.1 and 10.2 to 21.9 mg/gFW for glucose as well as from 6.2 to 35.5 mg/gFW and 17.4 to 47.5 mg/gFW for sucrose in normal and salinity conditions, respectively. Not only sucrose content constituted the largest amount of sugar in the melon fruits, but also influenced the most among the sugar compositions by salinity stress. Positive heterosis over the best parent (heterobeltiosis) was found for all the traits in stress conditions and for all traits with the exception of fructose in normal conditions. The Magasi × Gargar F_1 hybrid showed the highest heterobeltiosis for yield in both conditions. Considering means of yield, Rishbaba × Magasi showed the highest mean of fruit yield with positive heterbeltiosis under both conditions. Rishbaba × Sabouni exhibited the highest heterobeltiosis for total soluble solids and sucrose content of the fruits in stress. The current study demonstrated that the improvement of cultivars for fruits yield and quality is possible for both saline and normal conditions. It is, therefore, concluded that Rishbaba × Magasi and Rishbaba × Sabouni hybrids can be used to improve inbred lines in melon.

Target tomato breeding for special hydroponic technology

Irina T. Balashova, Sergey M. Sirota, Elena G. Kozar and Viktor F. Pivovarov

All-Russian Research Institute for Vegetable Breeding and Seed Production, Russian Federation

balashova56@mail.ru

Hydroponic technology on narrow benches is a new modular technology of vertical farming, which gives an opportunity to use vertical area of the greenhouse and to save energy and water resources. But the requirements for tomato forms, which will be cultivated by this technology, are very strict. So, we have developed the target technology of tomato breeding for special multi-circle hydroponic conditions. The first step was the development of the virtual model of the plant for the special hydroponic technology. On the whole, a virtual model of the plant represents a dwarf plant with high productivity, early ripening and resistance to the main stresses of greenhouses. The second step was the development of breeding strategy/ies for obtaining such forms of plants. We applied two known breeding technologies with our modifications. The first one was the screening of necessary forms from different tomato populations. *d*-genes, which control *dwarf*–trait in *Solanum lycopersicum* L., can appear at the seedling stage in the form of short internodes and short height of seedlings. So, we used the screening of short plants at the sporophyte stage - before flowering. We selected 57 dwarf samples of the 2 518 tomato samples for 3 years. And of the 57 dwarf samples we selected 2 samples only with the necessary characteristics for special hydroponics (#1 and # 46). We registered our priorities in obtaining them and F_6 of these forms are being tested at the state level now. The second breeding technology, which we used, was the hybridization. But we had some difficulties at this step of breeding. *d*-genes are recessive and will appear in F_2 –progeny only. Can we accelerate this process? Yes, we can, with the help of the target selection of parental forms. Using marker mutants collection (plants with *d*-genes and without it) and heritability coefficients, calculated by dispersion and correlation analyses, we showed, that *"the dwarf"*-trait (h^2=0,83) and *"the early ripening*"-trait (h^2=0,60) can be inherited on the paternal side. But the main traits of productivity - "*the mass of one fruit*" (h^2=0,99) and *"the number of fruit on the plant"* (h^2=0,96) - can be inherited on the maternal side (Balashova I.T. et al., 2014). So, we have selected 9 maternal and 7 paternal forms with necessary parameters for the target hybridization. Then we crossed maternal forms with good productivity and large fruit with *"dwarf's"* paternal forms, obtained F_1 – progenies and F_2 – progenies from self-pollinating of F_1 – progenies. F_2 –progenies were divided in the expected proportion: 3 parts of *"high"* plants and 1 part of *"dwarf"* plants. We selected some of early ripening plants with good productivity and large fruit from *"dwarf"* plants and studied F_3 – progenies of these *"dwarf"* plants. It was confirmed, that the strategy of the target hybridization was right: the height of the plant was lowered to the level of the *"dwarf's"* father. The mass of one fruit and the productivity of the plant have increased by 2 times. We have obtained some hybrid forms with the parameters needed the special hydroponics on narrow benches by now. And our researchers are going on.

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Development of molecular markers for sex expression in oriental melon (*Cucumis melo* L.)

Sun-Woong Bang[a] and **Sang-Min Chung[a]**

[a]*Life Science Department, Dongguk University, South Korea*

smchung@dongguk.edu

The DNA marker T1ex, originally developed from melon (*Cucumis melo* L.) for monoecy, was employed in chamoe, which is referred to as oriental melon. This marker shows size variations in monoecious melon. However, in chamoe, no such detrimental size variation was found in monoecious chamoe, and 99% association between flower phenotypes and genotypes of the T1ex marker was observed in 106 lines of chamoe. To evaluate the efficacy of the T1ex marker for marker-assisted selection (MAS), a total of 240 plants of chamoe breeding lines were screened using the T1ex marker. Among these, 98 varieties were selected. Although the T1ex marker might not be useful for MAS in melon, we found 100% concordance between genotypes and phenotypes for sex expression in chamoe. These results suggest that the T1ex marker will be a useful resource for MAS for monoecy in chamoe

Elites – a possibility to evaluate tomatoes landraces

Gallia Butnaru[a,b], Ioan Sarac[a] and Sorina Popescu[a]

[a] *Department of Genetics,*
Banat University of Agricultural Sciences and Veterinary Medicine, Timişoara, Romania
[b] *Academy of Romanian Scientists*

galliabutnaru@usab-tm.ro

Pre-breeding was carried out as individual selection of "Elites" within 6 landraces from different counties of Romania: Conop and Neagra from county Arad, Costeni318 from Maramures and Timbol from Timis. A variable number of Elite lines (ranging from zero to 14 per landrace) were investigated in field trials at Timisoara and Ineu.
The Elites varied greatly in plant size but this was correlated only weakly and insignificantly with root system growth. All elites had dense foliage, yellow corolla and occasionally pollen sterility was observed in dwarf plants. The immature fruits were light green, while the mature ones were red coloured. Many plants had a thick and rough exterior peel. The microscopic analysis revealed a thick and rugged epidermis as a plant response to stress conditions. The percentage of rough fruits in the different materials was around 50%, with segregation ratios fitting a χ^2 test for 1:1 of rough:smooth fruits in 20% of cases. Rugged fruit skin is a useful trait for long term fruit conservation in room temperature condition (which reached up to 3 months in elite E1973). The fruit shape varied from irregular flattened large fruits in all initial landraces to cylindrical, rounded, ellipsoidal and angular in the elites.
Fruit weight varied from 14.92±2.3g to 136±1.9g on Costeni 318 elites E01112 and E01012, respectively. The yield varied from 67 t/ha at the most precocious elite Strehaia 102 to 167 t/ha at Costeni dwarf elite E0312. The weight of 1000 seeds did not present significant differences among elites, being 3.11±058g and 3.75±1.22g in Neagra and Conop, respectively. In the unselected Costeni 318 landrace the lycopene (LYC) content varied from 0.65 μM/100 g to 3.05 μM/100g, with an average of 2.013±2.1 μM/100g and varied from 1.96 to 2.97 μM/100 g product in the elites, with an average of 1.86±3.7 μM/100g. Compared to Timisoara, at Ineu in the hilly area of Arad, the LYC content of the same genotype was repressed being 0.989±3.6 μM/100g in the former and 1.86±2.7 μM/100g in the latter.
The antioxidant capacity (FR) varied from 16 to 72.6 μM/100g FM for landraces and from 15.8 to 358 μM/100g FM for elites. The fruit of R46/3 plant has the highest soluble solids content (5.6%). In tomato juice of Costeni 318 elites the content of polyphenols, ascorbic acid and the antioxidant capacity (FR) were 143, 23 and 169 μM/100g respectively. Content of Ca, K, Mg and Fe had normal values (15.1, 1963.0, 151.0 and 16.44 ppm) and heavy metals were not found. The response of elites to acidic and basic salinity and to aluminium presence was satisfactory. The elites revealed a good tolerance to *Phytophthora* spp. (0.0 to 1.6) and sporadically plants and fruits affected by viral diseases were observed. The molecular similarity index emphasized high diversity. The selected and conserved elites are an indicator of the genetic value of tomatoes landraces collected in the Western part of Romania.

Morphological characterization of dihaploid altinbas melon (*Cucumis melo* L. var. *inodorus*) lines developed by haploidization technique

Baris Dal, Nebahat Sari and Ilknur Solmaz

Faculty of Agriculture/Department of Horticulture, University of Cukurova, Adana, Turkey

isolmaz@cu.edu.tr

Melon (*Cucumis melo* L.), is a member of *Cucurbitaceae* family and has a high level of morphological diversity in genus *Cucumis*. Significant genetic variation also exists in Turkey which is one of the gen centers of melon. Altinbas melons are the primary melon groups grown widely and economically in most regions of Turkey. These melons are odourless winter type melons belong to *Cucumis melo* var. *inodorus.* Their fruits are thick rinded and dark spotted on yellow rind. We developed 192 homozygous Altinbas melon lines using haploidization technique. These melon lines were grown in glass greenhouse in Department of Horticulture, Faculty of Agriculture, University of Cukurova and characterized for morphological characteristics according to the modified UPOV melon descriptor list for 68 characters (3 seedling, 3 plant, 9 leaf, 6 flower, 42 fruit and 5 seed). Data were analyzed by principle coordinate analysis (PCoA). Twenty quantitative characters (3 seedling, 3 plant, 3 leaf, 2 flower, 9 fruit) were also measured and subjected to principle component analysis (PCA). The results revealed that the Altinbas melon accessions have quite diversity for all the traits examined.

Selection and breeding of Valencian local tomato varieties

Maria R. Figàs[a], Dionís Borràs[a], Cristina Casanova[a], **Jaime Prohens[a]** and Salvador Soler[a]

[a] *Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Spain*

jprohens@btc.upv.es

The Mediterranean Old Kingdom of València (Spain) is home to a large number of local varieties of tomato (Figàs et al., 2015). These are classified in different varietal groups, with the 'Valenciana' and the 'De Penjar' being among the most prominent. The 'Valenciana' varietal group is characterized by presenting large, heart-shaped fruits with compact flesh and small locular cavities and few seeds. The fruits are highly appreciated and demanded locally for their excellent organoleptic properties and the demand has increased dramatically in the last years. One of the most renowned and demanded 'Valenciana' varieties is the 'Valenciana d'El Perelló', grown in sandy soils near the seaside. Regarding the 'De Penjar' type, this is a morphologically highly variable type (Figàs et al., 2015) characterized by the presence of the *alc* mutation that confers long shelf-life (Casals et al., 2012). The 'De Penjar' type is mostly used for rubbing it on bread. Among the 'De Penjar' varieties, the 'Tomata de Penjar d'Alcalà de Xivert' has a regional quality mark status. We have initiated a programme for the enhancement through selection and breeding of both the 'Valenciana d'El Perelló' and 'Tomata de Penjar d'Alcalà de Xivert'. Given that they have been traditionally conserved by farmers each of them present a certain degree of diversity. In consequence, we have initiated a programme of characterization of the materials grown by farmers and have made a selection of lines within each of these two local varieties. Selection criteria have included conformity with the ideotype of each of the local varieties, high yield, good fruit setting sequence and fruit size and shape uniformity. Selection has been done using a participatory approach in commercial fields of farmers that produce these types of tomato. As a result, we have selected a number of lines of 'Valenciana d'El Perelló' and 'Tomata de Penjar d'Alcalà de Xivert' with improved performance and characteristics. Also, as the 'Valenciana d'El Perelló' is susceptible to *Tomato mosaic virus* (ToMV), which causes a reduction of yield and quality, we have initiated a backcross breeding programme to introduce the *Tm2*^{2} resistance gene into this local variety. A commercial hybrid with a fruit morphology similar 'Valenciana d'El Perelló' variety has been used as donor parent. An intragenic *Tm2*^{2} SNP molecular marker is used for assisted selection in the backcross generations and selection for the 'Valenciana d'El Perelló' is performed in the ToMV resistant plants of the backcross generations. As a result of both programmes we are contributing to the enhancement of these tomato local varieties, which is of interest for the development of the local horticultural industry.

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Genetic diversity and population structure assessed by SSR in a large germplasm collection of pepper (*Capsicum* spp.)

Xiaozhen Gu[a], Zhenghai Zhang[a], Yacong Cao[a], Baoxi Zhang[a], Xixiang Li[ab] and **Lihao Wang**[a]

[a] *Key Laboratory of Vegetable Genetics and Physiology of the China Ministry of Agriculture, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, P. R. China*
[b] *National Gene Bank of Vegetables, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, P. R. China*

wanglihao@caas.cn

Deep understand of Germplasm is essential basis for breeding. 1904 accessions of pepper (*Capsicum* spp.) germplasms conserved by Chinese national vegetable gene bank were analyzed the genetic diversity and primary core collection using 29 SSR markers. Allele richness, gene diversity, PIC and index of heterozygosity were calculated. The gene diversity index and polymorphism information index respectively range from 0.016 to 0.883, 0.02 to 0.87 and respectively the average was 0.486 and 0.46. Among the 459 SSR alleles, 86 displayed a frequency of more than 5% in the total sample and hence were classified as 'common' alleles, while 81 displayed frequencies between 1% and 5% that were denoted as 'less common' alleles. Among the 292 remaining alleles, 159 were denoted as 'rare alleles' with frequencies between 0.1% and 1% and 133 as 'very rare' alleles with frequencies smaller than 0.1%.That 64% of the observed alleles showed a frequency of less than 1% in the mega-collection suggested that this collection represents the bio-diversity of pepper broadly. Analysis of population genetic structure, neighbor-joining tree and principal component showed that there are two different pepper genetic groups in China and admixtures are mostly located in between two populations. Group 1 contains 1411 accessions, characterized by triangular and horn shaped peppers. Its geographical distribution mainly concentrated in the southern region in our country, such as Hunan, Hubei, Sichuan, Yunan and Guizhou. Group 2 contains 493 accessions, characterized by large fruited peppers with blocky or rectangular shape, mainly distributed in the northern regions, such as Heilongjiang, Jilin, Liaoning and Hebei. M (M method) and R (Random method) predicted the optimal sample size of core germplasm. Two sampling methods showed that M score is higher than R ,no matter how sample size change, M method sampling alleles is significantly efficient than R method. M method was used to extract respectively 10,20,40,80 and 160 samples. Five sample sizes of core collection captured 28%,50%,57% and 69% alleles of raw materials. Considering the practicability of core collection, we selected characteristic accessions in different regions. The final core collection of 344 accessions (supplementary material) captured 81% of the SSR, including all common alleles,80(81)less common alleles,138(159)rare alleles and 68(133)vary rare alleles. Out of the 344 accessions,227 from group 1 and 117 from group 2.Its genetic diversity index and PIC index were 0.527 and 0.5 respectively, higher than those of raw materials(0.486,0.46).Using 29 SSR markers to analyze the neighbor-joining tree, result show that the final primary core accessions evenly distributed in raw materials and had high representative. The research contributes to understand the genetic background, kinship and geographical distribution between different groups. Pepper core collection of our Genebank is useful for reducing genetic redundancy and further gene development and utility.

Study of adaption and yield potential of 18 ecotypes of fennel in Bojnourd-Iran

Vahid Jajarmi and Afrooz Kolahdoozian

Isamic Aad University, Agronomy Department,Bojnourd Branch,Bojnourd,IRAN

vahid_jajarmii@yahoo.com

In order to evaluate the adaption to the Bojnourd climate and to determine the highest yielding varieties, 18 ecotypes of fennel (*Foeniculum vulgar mill*) were evaluated in an experiment conducted by RCBD with three replications during 2012-2014 in Islamic Azad University, Bojnourd Branch. Simple analyses showed that there was difference among ecotypes in parameters such as: seed yield, seed weight, planting inflorescence, planting time to flowering, number of seed per inflorescence. During two years, highest yield was observed for ecotype no.15 (1082 kg/ha). The most days from planting to flowering and maturity were observed for No18 (Kashan ecotype) and shortest time from flowering to maturity was attributed to ecotypes 6, 5 and 4. The highest seed yield per inflorescence belonged to ecotype no 14 (Ebnesina-Hamedan). Combined analysis of variance showed significant differences (P<%0) among ecotypes, environment and interactions (G*E). Stability analyses were done by non-parametric rank, AMMI methods. The results of evaluated methods were the comparable. Based on these results, 3 ecotypes with high stability and grain yield were selected: 10 (seeds collected from Yazd 1), 14 (seeds collected from Ebnesina-Hamedan) and 15 (Europe). These ecotypes can be successfully grown in the testing location and other regions with similar climatic conditions.

In vitro polyploidy induction in Persian endemic *Papaver bracteatum* (Papaveraceae)

Ghasem Karimzadeh[a], Saeed Tarkesh Esfahani[a] and Mohammad Reza Naghavi[b]

[a] *Department of Plant Breeding and Biotechnology, Tarbiat Modares University, Iran*
[b] *Department of Agronomy and Plant Breeding, University of Tehran, Iran*

karimzadeh_g@modares.ac.ir

Persian poppy (*Papaver bracteatum* Lindl.) is a wild perennial medicinal plant that grows natively in the Alborz Mountains in Northern Iran. It is mainly known for the high amounts of the pharmaceutically valuable alkaloid of thebaine. The *in vitro* production of autotetraploid *P. bracteatum* by colchicine treatment of newly germinated seeds is being reported for the first time. Tetraploid and mixoploid progenies were quickly and effectively confirmed by either flow cytometric technique or microscopic chromosome counting. The chromosome number in diploids and induced tetraploids were verified to be $2n = 14$ and $2n = 28$, having 2C DNA amounts of 6.15 ± 0.03 and 11.95 ± 0.07 pg, respectively. The highest tetraploidy induction efficiency was 31.3%, yielded at a colchicine concentration of 0.05% and treatment duration of 24 h. Colchicine concentration was considered as the most important determining factor in successful *in vitro* polyploidy induction. Both ploidy induction efficiency and the toxic effects of colchicine on plant survival and growth were proportional mainly to its concentration rather than the exposure duration. Tetraploid plants had significantly larger and less frequent leaf stomata, comprising larger cells size. These morphological traits may serve as reliable criteria for preliminary ploidy level screening of *P. bracteatum* populations.

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Environmental factors affecting *Bemisia tabaci* resistance of tomato

Atiyeh Kashaninia[a,b], Adriaan W. van Heusden[a], Rene A.A. van der Vlugt[c], Richard G.F. Visser[a] and Ben Vosman

[a] *Wageningen UR Plant Breeding, Wageningen, The Netherlands.*
[b] *Graduate School Experimental Plant Sciences, Wageningen, the Netherlands.*
[c] *Wageningen UR Biointeractions and Plant Health, Wageningen, The Netherlands.*

atiyeh.kashaninia@wur.nl

Biotic and abiotic stresses are limiting factors in crop production. Plants can be exposed to various stresses concurrently, which may either enhance or decrease the effect of the individual stress. *Bemisia tabaci* is a phloem feeding insect that can affect tomato yield by depleting the plant from photo- assimilates, wilting of leaves, and covering leaves with honey dew on which molds may grow, but the main damage inflicted by the whitefly is caused by the devastating viruses it transmits. Some wild tomato species possess morphological and metabolic adaptations to resist whitefly infestation. Glandular trichomes type I and IV are the first layer of defense, covering the aerial parts of the plants. They have a production, storing and secretory system for specialized secondary metabolites. These trichome types are absent on cultivated tomato, which contain amongst others trichomes of type III and V. These non-glandular trichomes are similar to I and IV except for the glandular head. Trichome type IV production and longevity can be influenced by environmental factors like water and salt stress, light or heat stress and biotic stresses which in turn can affect whitefly resistance levels. We studied the effect of single stresses on glandular trichome type IV and non-glandular trichome type V production as well as whitefly resistance of tomato. A moderate salt stress and six phytohormones were used on *Solanum galapagense*, *Solanum lycopersicum* and an F_1 hybrid between these species. In addition, we also studied the effect of ToMV (Tomato Mosaic Virus) infection as a model system for biotic stress. The results will be discussed and show that SA, JA, Cytokinin, Ethylene, Salinity and ToMV affect trichome density and whitefly resistance in these plants.

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Single nucleotide polymorphisms linked to *SlMYB12* gene that controls fruit peel color in domesticated tomatoes (*Solanum lycopersicum* L.)

Bichseam Kim, Namhui Kim, Younghoon Park and **Beung-Gu Son**

Department of Horticultural Bioscience, Pusan National University, Miryang, Korea

bgson@pusan.ac.kr

Yellow or transparent fruit peel color is caused by the accumulation or lack of naringenin chalcone (NG, C) in fruit peel and determines the red or pink appearance of tomato fruit, respectively. NGC synthesis is regulated by the *SlMYB12* gene of the *Y* locus on chromosome 1, and DNA markers derived from *SlMYB12* can be useful for marker-assisted selection (MAS) of tomato fruit color. To develop a gene-based marker, a 4.9 kb of the *SlMYB12* gene including a potential promoter region was sequenced from the red-fruited (*YY*) line 'FCR' and pink-fruited (*yy*) line 'FCP'. Sequence alignment of these *SlMYB12* alleles revealed no sequence variations between 'FCR' and 'FCP'. To identify *SlMYB12*-linked single nucleotide polymorphisms (SNPs), 'FCR' and 'FCP' were genotyped using a SolCAP Tomato SNP array and CAPS markers (CAPS-456, 531, 13762, and 38123) were developed from the four SNPs (solcap_snp_sl_456, 531, 13762, and 38123) most closely flanking the *SlMYB12*. These CAPS markers were mapped using F_2 plants derived from 'FCR' × 'FCP'. The map positions of the fruit peel color locus (*Y*) were CAPS-13762 (0 cM) - 456 (11.09 cM) - *Y* (15.71 cM) - 38123 (17.82 cM) - 531 (30.86 cM), and the DNA sequence of *SlMYB12* was physically anchored in the middle of #456 and #38123, indicating that fruit peel color in domesticated tomato is controlled by *SlMYB12*. A total of 64 SolCAP tomato germplasms were evaluated for their fruit peel color and SNPs located between solcap_snp_sl_456 and 38123. Seven SNPs that were detected in this interval were highly conserved for pink-fruited accessions and specific to transparent fruit peel traits, as depicted by a phenetic tree of 64 accessions based on the seven SNPs.

Development of a high-resolution melting marker for selecting Fusarium crown root rot-resistance in the tomato

Bichsaem Kim[a], Jihyunh Wang[a], Joonyoung Kim[b], Byungsup Kim[b], Sungran Min[c], Younghoon Park[a] and **Jumsoon Kang[a]**

[a]Department of Horticultural Bioscience, Pusan National University, Korea
[b]Department of Plant Science, Gangneung-Wonju National University, Korea
[c]Plant Systems Engineering Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Korea

kangjs@pusan.ac.kr

Fusarium crown root rot is a severe fungal disease caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) in tomato. In this study, genomic location of the FORL-resistance locus was assessed using a set of molecular markers physically anchored on Chr. 9 and an F_2 population derived from FORL-resistant inbred AV107-4 (*Solanum lycopersicum*) × susceptible L3708 (*S. pimpinellifolium*). Bioassay of the F_2 populations with Korean FORL strain KACC 40031 showed single-dominant inheritance of FORL resistance. A total of 13 PCR-based markers encompassing approximately 3.6–72.0 Mb of Chr. 9 were developed based on the Tomato-EXPEN 2000 map and SolCAP Tomato SNP array analysis. These markers were genotyped on 345 F_2 plants, and the results indicated that the FORL-resistance locus is present on a pericentromeric region of suppressed chromosomal recombination in Chr. 9. FORL resistance locus was further localized by testing these markers against diverse commercial tomato and stock cultivars resistant to FORL. A restriction fragment length polymorphism (RFLP) marker, PNU-D4, located on ~6.1Mb demonstrated the highest match with the resistance, and was converted to a high-resolution melting (HRM) marker for marker-assisted selection of FORL resistance.

Major quantitative trait loci and putative candidate genes for powdery mildew resistance and fruit-related traits revealed by an intraspecific genetic map for watermelon (*Citrullus lanatus* var. *lanatus*)

Kwanghwan Kim[a], Jihyun Hwang[a], Dongyeup Han[a], Minkyu Park[b,c], Seungill Kim[b], Doil Choi[b], Yongjae Kim[d], Gungpyo Lee[e], Suntae Kim[f] and **Younghoon Park[a]**

[a] *Department of Horticultural Bioscience, Pusan National University, Korea*
[b] *Department of Plant Science, Seoul National University, Korea*
[c] *Department of Genetics, University of Georgia, U.S.A.*
[d] *Partner Seed, Ansung, Korea*
[e] *Department of Integrative Plant Science, Chung-Ang University, Korea*
[f] *Department of Plant Bioscience, Pusan National University, Korea*

ypark@pusan.ac.kr

An intraspecific genetic map for watermelon was constructed using an F_2 population derived from 'Arka Manik' × 'TS34' and transcript sequence variants and quantitative trait loci (QTL) for resistance to powdery mildew (PMR), seed size (SS), and fruit shape (FS) were analyzed. The map consists of 14 linkage groups (LGs) defined by 174 cleaved amplified polymorphic sequences (CAPS), 2 derived-cleaved amplified polymorphic sequence markers, 20 sequence-characterized amplified regions, and 8 expressed sequence tag-simple sequence repeat markers spanning 1,404.3 cM, with a mean marker interval of 6.9 cM and an average of 14.6 markers per LG. Genetic inheritance and QTL analyses indicated that each of the PMR, SS, and FS traits is controlled by an incompletely dominant effect of major QTLs designated as *pmr2.1*, *ss2.1*, and *fsi3.1*, respectively. The *pmr2.1*, detected on chromosome 2 (Chr02), explained 80.0% of the phenotypic variation (LOD = 30.76). This QTL was flanked by two CAPS markers, wsb2-24 (4.00 cM) and wsb2-39 (13.97 cM). The *ss2.1*, located close to *pmr2.1* and CAPS marker wsb2-13 (1.00 cM) on Chr02, explained 92.3% of the phenotypic variation (LOD = 68.78). The *fsi3.1*, detected on Chr03, explained 79.7% of the phenotypic variation (LOD = 31.37) and was flanked by two CAPS, wsb3-24 (1.91 cM) and wsb3-9 (7.00 cM). Candidate gene-based CAPS markers were developed from the disease resistance and fruit shape gene homologs located on Chr.02 and Chr03 and were mapped on the intraspecific map. Colocalization of these markers with the major QTLs indicated that watermelon orthologs of a nucleotide-binding site-leucine-rich repeat class gene containing an RPW8 domain and a member of SUN containing the IQ67 domain are candidate genes for *pmr2.1* and *fsi3.1*, respectively. The results presented here in provide useful information for marker-assisted breeding and gene cloning for PMR and fruit-related traits.

Development of DNA markers associated with monoecious sex expression in melon (*Cucumis melo* L.)

Nahui Kim[a], Juyeol Oh[b], Bichseam Kim[a], Jack E. Staub[c], Sang-Min Chung[d], Younghoon Park[a] and **Young-Guen Lee**[a]

[a]*Department of Horticultural Bioscience, Pusan National University, Miryang, Korea*
[b]*Gyeongnam Agricultural Research & Extension Services, Jinju, Korea*
[c]*U.S. Department of Agriculture, Agricultural Research Service, Forage and Range Research Laboratory, Logan, UT, U.S.A.*
[d]*Department of Life Science, Dongguk University-Seoul, Seoul, Korea*

lyg5354@pusan.ac.kr

Most melon (*Cucumis melo* L.) breeding lines in South Korea display andromonoecious sex expression, which necessitates laborious hand emasculation during F_1 hybrid seed production. Thus, there is a need to develop monoecious sex types in elite germplasm to obviate self-pollination. Sex expression is associated with floral ethylene production, which, in monecious melon plants, is associated with the *A* locus. Our study was conducted to develop molecular markers for selection of monoecious plants based on sequence variation inherent in the *CmACS-7* gene [1-aminocyclopropane-1-carboxylic acid synthase (ACS) activity] that is associated with ethylene production. Full-length *CmACS-7* sequences were cloned from a monoecious (MO23) and andromonoecious (AM24) line. The alignment of those *CmACS-7* sequences revealed a single nucleotide polymorphism (SNP; C170T) in exon 1 and an 18-bp indel in the 3′-untranslated region (UTR) of between MO23 and AM24, which was then used to develop a cleaved amplified polymorphic sequence (CAPS) (EX1-C170T) and sequence characterized amplified region (SCAR) marker (T1ex) from the SNP and indel, respectively. The sex expression and the T1ex SCAR-based genotype of 442 F_2 plants derived from a line MO23 × AM24 cross was determined. Monoecy and andromonoecy segregated in a 3:1 ratio in F_2 progeny, where the sex type of 429 plants (13 plants not classified) co-segregated with the SCAR marker, demonstrating that sex expression regulated by *CmACS-7* is controlled by a single dominant gene and that it confers monoecy in line MO23. Allelic variation in 112 geographically diverse melon lines for *CmACS-7* as accessed by CAPS EX1-C170T and SCAR T1ex markers indicated that the: 1) exon 1 of *CmACS-7* is highly conserved and the SNP/sex expression association detected is highly predictable making it potentially useful for marker-based selection of monoecious plants, and; 2) 18-bp indel mutation in the 3′-UTR was present in various lengths depending on different monoecious melon germplasm.

Anti-VEGFR2 nanobody expression in lettuce using an infectious *Turnip mosaic virus* vector

Malihe Mirzaee[a], **Mokhtar Jalali-Javaran[a]**, Ahmad Moieni[a], Sirous Zeinali[b], Mahdi Behdani[c], Masoud Shams-Bakhsh[a], Mostafa Modarresi[a]

[a] *Department of Plant Breeding & Biotechnology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran*
[b] *Department of Molecular Medicine, Pasteur Institute of Iran, Tehran, Iran*
[c] *Biotechnology Research Center, Biotechnology Department, Venom & Biotherapeutics Molecules Lab., Pasteur Institute of Iran, Tehran, Iran*

m_jalali@modares.ac.ir

Angiogenesis plays an important role in tumor growth and metastasis of cancer and vascular endothelial growth factor (VEGF) is the key regulator in stimulating angiogenesis. The VEGF activity is mediated by binding to its cell-surface receptors, mainly VEGFR2. Therefore, inhibition of the VEGF/VEGFR2 interaction by antibodies is investigated as a therapeutic strategy in cancer therapy. Here, we describe transient expression of an anti-VEGFR2 nanobody (3VGR19) by a viral vector based on *Turnip mosaic virus* (TuMV) in lettuce (*Lactuca sativa* L.). RT-PCR analysis demonstrated the 3VGR19 transcript expression. Western blot analysis showed the 3VGR19 protein expression with an expected molecular mass of ~15 kDa and based on the ELISA results, the expression level of 3VGR19 was 8 µg/g of leaf fresh weight. Taken together, recombinant 3VGR19 could be efficiently expressed in lettuce leaves and TuMV-based expression system would be an appropriate platform for production of recombinant proteins in lettuce plants.

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Identification of cytosine-5 DNA methyltransferases and demethylases in eggplant and their expression during fruit ripening

Andrea Moglia, Silvia Gianoglio, Alberto Acquadro, Danila Valentino, Anna Maria Milani, Sergio Lanteri, Cinzia Comino

DISAFA, Plant Genetics and Breeding, University of Torino, Italy

sergio.lanteri@unito.it

DNA methylation is a major epigenetic modification which plays important roles in genome protection and regulation of gene expression during developmental processes.
In plant DNA methylation occurs in all sequence contexts (CpG, CpHpG, and CpHpH, where H is adenine, cytosine, or thymine) and is regulated by both C5-MTase and demethylases. The former includes methyltransferase (MET), chromomethyltransferase (CMT) and domains rearranged methyltransferase (DRM). MET and CMT are respectively responsible for the maintenance of CpG and CpHpG methylation, while DRM for the maintenance of CpHpH methylation as well as for *de novo* methylation.
By exploiting the available genome resources and protein sequences of Arabidopsis and tomato, we identified six C5-MTases (1 MET, 3 CMTs and 2 DRMs) and one demethylase (DME1) in eggplant (*Solanum melongena* L.) genome, and assessed their transcript abundance during fruit ripening in four cultivars.
The expression level of MET1, DRM1 and DRM2 increased with the progress of ripening. An analogous expression pattern was observed for CMT2 and CMT3, while a reduction of transcript abundance was detected in CMT1 as well as in the demethylase DME1.
The comprehensive gene expression analyses of C5-MTases in eggplant provide evidence of their key role during the berry maturation, which is a complex developmental process including modification of tissue firmness, change in sugar metabolism as well as variation in both composition and levels of secondary metabolites.

Identification of heterotic groups in summer squash (*Cucurbita pepo* L.) through morphologic and molecular methods and heterosis in agronomic characteristics

Cetin Nacar[a], **Nebahat Sari[b]** and Nedim Mutlu[c]

[a] *Alata Horticultural Research Institute/Department of Breeding, Turkey*
[b] *Faculty of Agriculture/Department of Horticulture, University of Cukurova, Turkey*
[c] *Faculty of Agriculture/Department of Horticultural Biotechnology, University of Akdeniz, Turkey*

nesari@cu.edu.tr

Heterosis is positively correlated with the degree of dissimilarity in the gametes. The objective of the study was to assess heterosis from the data obtained from morphologic and molecular characterizations of the lines in summer squash. This study was conducted at the Alata Horticultural Research Institute, Erdemli- Mersin, Turkey in 2012 and 2013. Heterosis was assessed in hybrids created from crosses involving parental lines predicted to be at different heterotic groups. The hybrids were grown in open fields and under cover during the spring and autumn seasons. In trials, seedling and plant measurements, time to male and female flower bloom, yield and fruit characteristics were measured and heterosis were calculated. The high positive heterosis were observed in early and total yields. Mean heterosis levels in early yield ranged from 22% to 663%. Heterosis in total yield varied between 28% to 220%. The results indicate that heterotic groups could be generated through the use of molecular markersand morphological data and high heterosis levels could be achieved especially for early and total yield.

New germplasm resources and genetic variation of *Cucumis melo* var. *flexuosus* (Faqqous)

Samer Omari[a], Omar B. Mallah[b], Rana M. Jamous[b], Mohammed S. Ali-Shtayeh[b] and **Rafael Perl-Treves**[a]

[a]*Faculty of Life Sciences, Bar Ilan University, Israel*
[b]*Biodiversity and Environmental Research Center, BERC, Palestine*

Local traditional varieties (landraces) represent invaluable genetic resources for crop breeding. Landraces of elongated, cucumber-like melons of ancient domestication, called snake melon, or Faqqous, are grown in the open, rain-fed field on significant scale in Palestinian and Israeli Arab villages, where they exhibit good climatic adaptation, and some stress and disease tolerance traits. Fruits are picked about one week after anthesis and eaten immature like cucumbers, and their downy fruits are appreciated for their rich taste. It is highly popular also in other Mediterranean countries such as Southern Italy, Turkey, Syria and Egypt. In a Palestinian-Israeli collaborative project funded by MERC-USAID, an extensive collection of Faqqous landraces was assembled and described, including four main horticultural types (White Baladi, Green Baladi, White Sahouri and Green Sahouri). Morphological variation was recorded in a Common Garden experiment. Yield components and traits that are potentially related to yield, such as degree of femaleness and senescence time course, were measured and between-traits correlations were calculated. Representative DNA samples were analyzed by Diversity Array Technology (DArT), a high throughput genotyping method, revealing high levels of diversity and heterozygosity in the collected samples. Diversity is being analyzed with respect to morphological variation, geographic and climatic variables, to understand population structure and identify key traits that could be targeted for future improvement of Faqqous for local farmers.

Heritability of artichoke important traits in hybrids developed by a USA-Italy agreement

Mario A. Pagnotta[a], Nestor A. Rey[b], Linda Mondini[a], Rusty Jordan[b] and Francesco Saccardo[a]

[a] *Department of Science and Technologies for Agriculture, Forestry, Nature and Energy (DAFNE), University of Tuscia, Viterbo, Italy.*
[b] *2Big Heart Seed, California 92227, USA*

pagnotta@unitus.it

Nowadays hybrids in globe artichoke (*Cynara cardunculus* L. subsp. *scolymus*), even if not Mendelian F1 reach a good uniformity. Hybrid seed-propagated artichoke solves several negative aspects caused by the micro-vegetative propagation of the plants. Seeds are cheap, easily to manage, virus free, storable, etc. Hybrid uniformity together with quality characteristics is one of the most important tasks; in fact, the parents used in the crosses could be not highly homozygous due to the inbreeding depression affecting the plants. Uniform hybrids with promising quality and agronomic characteristics, to be introduced for fresh consumption and industry processes, have been obtained under the cooperation between University of Tuscia (Italy) and Big Heart Seed (California – USA). By analyzing the parents and the hybrid characteristics the heritability of important agronomic treats have been computed and here reported. The differences between maternal and paternal heritability is also reported and discussed. Hybrids have been also characterized by molecular markers to assess their genetic distance and parents' effects.

Moreover, the results of germplasm material evaluation performed under two different environments: i.e. the Imperial Desert of California in Brawley (33°02'48" 115°31'43"W) and the Italian Mediterranean environmental conditions of Central Italy (Rome) (41°57'29"N 12°07'10"E) are reported. Evidence indicated that, in spite of the high heterozygosity present in parent lines, there is a possible combination of the traditional morphological evaluation with the use of molecular markers to classical genetic analysis in predicting and achieving hybrids constitutions and to evaluate their heritability and hence the probability to have that particular trait in the progeny. The morphological evaluations run in USA and in Italy, show consistence in spite of the great environmental differences between the two sites

Genome-wide sequence variants and their implication for breeding in watermelon

Girim Park[a], Bingkui Kim[a], Yunsung Kim[b], Huibum Yang[b], Sungwoo Park[b], Gungpyo Lee[c], Younghoon Park[a] and **Yongjae Lee[a]**

[a] *Department of Horticultural Bioscience, Pusan National University, Korea*
[b] *NH Seed, Ansung, Korea*
[c] *Department of Integrative Plant Science, Chung-Ang University, Korea*

yjl@pusan.ac.kr

An array of watermelon (*Citrullus lanatus* var. *lanatus*) accessions comprised of 19 elite inbred lines with diverse phenotypic traits were collected from private seed companies. The whole genomes of these cultivars were resequenced using a HighSeq 2000 and NextSeq (Illumina) platform. Raw sequence data more than 20-fold of the watermelon reference genome size (350 Mb) were obtained by paired-end sequencing (2 × 100~150 bp) from each genomic library. The number of homozygous single nucleotide polymorphism (SNP) and insertion/deletion (indel) varients ranged from 81,776~193,491 and 6,497~13,404 in this collection, respectively. SNP-based Principle component analysis (PCA) and Bayesian clustering of 19 elite inbred lines were conducted based on the distribution of genome wide diversity of sequence variants. Allele abundance specific to cultivar type (fruit skin color, fruit shape, stripe pattern, flesh color) was investigated. Linkage disequilibrium (LD) analysis was carried out using Haploview to identify LD block on each chromosome and QTL region. In addition, a set of SNPs with high PIC value was selected as potential markers for Marker-assisted backcross (MAB). This study provided information for genetic structure and implication for breeding in modern watermelon cultivars.

The whole genome microsatellite database of eggplant

Ezio Portis[a], Lorenzo Barchi[a], Laura Toppino[b], Flavio Portis[c], Luisa Valente[c], Giuseppe Leonardo Rotino[b], Sergio Lanteri[a] and Alberto Acquadro[a]

a DISAFA, Plant Genetics and Breeding, University of Torino, Italy
b CREA-ORL, Montanaso Lombardo (Lodi), Italy
c Yebokey, Torino, Italy

lorenzo.barchi@unito.it

Microsatellites or simple sequence repeats (SSRs) occur ubiquitously in all prokaryotic and eukaryotic genomes and are present in both coding and non-coding regions.. They represent one of the most informative, versatile and practical DNA-based markers used in plant breeding programs, since they are easy to score and have wide genomic distribution, codominant inheritance and a multiallelic nature. Microsatellite development has traditionally been a time consuming and costly process, however, the availability of whole genome sequences and *in silico* approaches has revolutionized bulk SSR marker discovery.

Perfect, imperfect and compound SSRs were *in-silico* mined using the *SciRoKo SSR-search* module (http://kofler.or.at/bioinformatics/SciRoKo/) within pseudomolecules as well as unmapped scaffolds of the recently developed high quality reference eggplant (*Solanum melongena* L.) genome (see Barchi et al. 2016, present Congress). Any sequence was considered as an SSR where a motif, of a minimum length of 15 nucleotides, was repeated at least 4 times; accordingly any sequence was considered as a perfect SSR whenever a motif was repeated at least 15 times (1nt motif), eight times (2nt), five times (3nt) or four times (4-6nt), allowing for only one mismatch. For compound repeats, the maximum default interruption (spacer) length was set at 100bp.

From the ~1.2 Gb of eggplant genomic sequence, we identified 133,602 perfect SSR motifs (density of 113.59 SSR/Mb), which included 20,760 (15.5%) compound SSRs. The imperfect SSR motifs identified were nearly 179,000. A series of 352 motif types was detected, which ranged from mono- to hexa-nucleotides and among which dominated di-nucleotide (42.5%) and tri-nucleotide repeats (36.8%). The density of mono-, tetra-, penta- and hexa-nucleotide repeats was 8.3%, 7.1%, 2.8% and 1.9% respectively. A/T, AT/AT, AAC/GTT, AAAT/ATTT, AAAAT/ATTTT and AACAAT/ATTGTT were the most frequent repeats among mono- to hexa-nucleotide SSRs. The microsatellite density was found to vary among the 12 eggplant chromosomes, being the highest on ch. E09 (166.56 SSR/Mb) and the lowest on ch. E12 (116.87 SSR/Mb).

In order to assist genetic studies and breeding applications, SSRs have been classified and made accessible through an user friendly tool named *EgMiDB* (*Eggplant Microsatellite Database* - http://www.eggplantmicrosatellite.org). This freely accessible web application, based on a LAMP solution stack, organizes microsatellites data in a MySQL database and provides an effective and responsive interface developed in PHP. Search for SSRs may be customized by limiting their location on a chromosome as well as number of markers in that range. To cater the customized needs of wet lab, this database has been further implemented with *Primer3* for primer designing of selected markers, with left and right size of flanking regions up to 200 bp; this enable researchers to select markers of choice at desired interval over each chromosome. *EgMiDB* database allows to select SSRs of particular type or from a specific region in the genome and is expected to provide a key contribution to both basic genomics research and breeding applications in eggplant as well as other Solanaceae crops.

Understanding hybrid seed failure in wild tomatoes: phenotypic and transcriptomic signatures

Morgane Roth[a], Ana M. Florez-Rueda[a], Stephan Griesser[a], Markos Tsinganis[a], Margot Paris[a] and Thomas Städler[a]

[a]*Plant Ecological Genetics, Institute of Integrative Biology, ETH Zurich, Switzerland*

morgane.roth@env.ethz.ch

The introgression of interesting traits from wild relatives to crops is a common practice in plant breeding. However, crossing barriers between species are often problematic as they may prevent the formation of viable or fertile hybrids. Here we focus on hybrid seed failure in wild tomatoes, characterized by endosperm dysfunction leading to embryo abortion. Crosses between *Solanum peruvianum* and either *S. chilense* or *S. arcanum* var. marañón result in complete hybrid seed failure ("strong barrier"), while crosses between *S. chilense* and *S. arcanum* var. marañón yield substantial seed survival and subsequent germination ("soft barrier"). To understand the molecular mechanisms involved in these phenotypes, we conducted a large RNA-Seq experiment with three intra-specific and three inter-specific reciprocal crosses involving the aforementioned species. Endosperm tissue was sampled at 12 days after pollination by laser microdissection and Illumina-sequenced. Our transcriptomic data analysed at the gene level with both total read counts and parent-specific allelic counts show i) a shift towards increased maternal expression in hybrid crosses, where genes tend to behave in the same way when considering two hybrids sharing the same mother, ii) a higher shift toward maternal expression in the "strong barrier" hybrids, and iii) a less extreme pattern in the "soft barrier" hybrids with very moderate expression changes compared to intra-specific crosses. Representatives of these latter hybrids are currently grown in our greenhouse to characterize their growth and reproductive abilities. All together, our data highlight the complexity of postzygotic reproductive isolation and will provide candidate genes to uncover common molecular mechanisms of hybrid seed failure.

Exploitation of diversity in cultivated lettuce through genome wide association analysis

Emidio Sabatini[a], Franco Donati[c], Massimiliano Beretta[c], **Daniela Palma[a]**, Alessandro Tondelli[b] and Luigi Cattivelli[b]

[a] *CREA-ORA, Council for Agricultural Research and Economics, Research Institute for Vegetable Crops, Italy*
[b] *CREA-GPG, Council for Agricultural Research and Economics, Genomics Research Center, Italy*
[c] *ISI Sementi Spa, Italy*

danielapalma82@gmail.com

In the current study, we focused on distilling the molecular diversity and genetic structure of 141 homozygous *Lactuca sativa* lines belonging to 8 horticultural types, using this information to assess genome-wide marker-trait associations.
The following types are represented in the collection: Butterhead, Batavia, Crisphead, Leaf, Oilseed, Stem, Cos, Oakleaves. The varieties were chosen from the CGN germplasm collection and from the most known seed companies catalogues, according to their genetic background in terms of resistance to pests and disease and variability of the main phenotypic traits.
A comprehensive, highly informative and well-distributed set of markers was generated through RAPiD-Seq technology (a modification of standard GBS approach). A total of 24,327 polymorphic molecular markers were obtained, representing one of the richest set of markers available so far in lettuce.
Association tests were performed between the molecular markers and four phenotypic traits (*Nasonovia ribisnigri* resistance, seed coat colour and bolting delay/speed) representing characters with different genetic basis and distribution across the germplasm analyzed.
The assessment of the population structure was conducted by principal coordinates analysis (PCoA) using the software PAST and by a Bayesian clustering approach with STRUCTURE. This allowed the identification of five sub-populations, which fits well with the most important lettuce typologies as reported in previous phylogenetic studies.
Association analysis was performed using the software GAPIT and three linear models with or without covariates related to kinship and/or populations structure (GLM, CMLM + K and CMLM+Q+K); the markers were indicated as significantly associated after a False Discovery Rate (FDR) filtering. To test the models and to assess the reliability of the whole system, both monogenic and quantitatively inherited traits were considered.
Monogenic traits such as *Nasonovia* resistance and seed coat colour were correctly imputed to single genomic locations with strong association values.
For quantitatively inherited traits, such as bolting delay (q) and bolting speed (m), the phenotyping was carried out in 3 locations for 2 years and the datasets were used separately *per* single location and *per* year. Using both linear model and covariate, a clear association was found for both bolting parameters (q and m) with a set of three markers closely related and located on chromosome 7. The sequences including these SNPs match with the sequence of the *Lactuca sativa* flowering locus T (FT) gene (GenBank: JX485630.1). These results confirm the robustness of this new association mapping resource in lettuce.

Identification of candidate genes involved in plant defense response against some important potato viruses using microarray meta-analysis

Mohammad Sadegh Sabet[a], Zhila Osmani[a] and Masoud Shams-Bakhsh[b]

[a]*Department of Plant Breeding and Biotechnology, Tarbiat Modares University, Tehran, Iran*
[b]*Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran*

ms.sabet@modares.ac.ir

Potato (*Solanum tuberosum* L.) is the most fourth important food crop worldwide following wheat, rice and maize that grown in approximately 80% of the countries across the world with an annual production in excess of 330 million tons per year. Viruses are among the most agriculturally important groups of plant pathogens, causing serious economic losses in many major crops by reducing yield and quality. PVY, PVA and PLRV have gained attention as devastating viruses in potato farming. In recent years, microarray technology has been widely used for identification of differentially expressed genes. Microarray meta-analysis methods allow integration of a potentially large number of datasets and represent new insights into gene regulation that may have been overlooked or not detected in single experiments. This study has taken advantage of meta-analysis of microarray data sets to identify robust sets of genes regulated by interaction between potato plants and viruses, to identify genes up-regulated in different conditions and to select the most important genes. For this purpose, microarray data was collected from gene expression databases such as GEO and ArrayExpress. All genes with a 1.25-fold change or more and *P*-value of 0.05 or less in plants challenged with viruses were selected for each dataset based on Fisher's test, resulting in a list of differentially expressed genes. Data filtering was carried out by comparing resistant and susceptible host datasets and combining these with data derived from microarray experiments involving biotic (bacterial and fungal infections, nematode and insect attack) and abiotic (cold, heat, salt and drought) stresses. The functional categorization, biological functions and metabolic pathways of candidate genes were also annotated using J-Express 2012 and MapMan 3.5.1R2 softwares. These results showed that nearly half of the genes were involved in plant response to viral infection also related to other stresses. Finally, 42 genes were identified that specifically responded to the virus infection and 265 genes were also recognized to respond either in virus infection or other stresses. The selected genes were involved in plant processes of photosynthesis, perception, signaling and defense response. As expected, integration of gene expression information from microarray studies using meta-analysis can help to characterization of the complex biological processes transcriptome.

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A preliminary analysis of onion (*Allium cepa* L.) genetic diversity in Europe

Cristina Silvar

Departamento de Bioloxía Animal, Bioloxía Vexetal e Ecoloxía, Universidade da Coruña, Spain

c.silvar@udc.es

The assessment of genetic diversity within crops results of pivotal importance for a sustainable agriculture. In this context, landraces, i.e. native varieties empirically selected by farmers over time and well adapted to specific agro–climatic represent valuable reservoirs of wide genetic diversity. Onion (*Allium cepa* L.) is one of the most widely cultivated vegetables around the world. The economic value of this crop derives from its culinary usages, nutritional benefits and health–giving properties. Europe produces almost 15% of the total commercialized onion worldwide, the Netherlands and Spain being the principal producing countries. In spite of such significance, the characterization and exploitation of novel onion genetic resources have been limited and primarily hampered by the out–breeding and biennial habit of the crop as well as by the paucity of large sets of robust molecular markers. Hence, those research works has been exiguous in Europe and mainly centered in agro–morphological characterizations and analyses of organoleptic traits.

In the present work, a collection of 96 genotypes representing 16 onion European landraces from Spain, Portugal, France, Italy, Germany, Bulgaria, Netherlands, Hungary and United Kingdom, togehter with an outgroup from Tunisia, were genetically assessed with a set of 25 genomic and EST-based microstallite markers. Twenty markers were informative, provinding a total of 104 alleles, with an average value of 5.2 alleles per locus. The observed heterozygosity (H_o) values were high and close to 0.50 for the majority of landraces. The H_e and uH_e ranged from 0.26 and 0.28, respectively, to 0.55 and 0.60, with a mean value of 0.62 over the whole set of European onions. Pairwise similarities among landraces were computed using the Nei´s genetic distance, which ranged from 0.193 to 0.848, with an average of 0.431. Genetic discrimination among individuals and landraces was performed through a factorial correspondence analysis, which showed that plants within the same accession tend to cluster in small areas of the plane, suggesting the existence of real groups of landraces. A pattern of clustering based on the geographical origin was not clearly detected, although some trends could be observed. Thus, landraces from the Iberian Peninsula (Spain and Portugal) and those from Central Europe (Germany and Netherlands) tended to group together. A Bayesian analysis with the software STRUCTURE produced a most probable value of K=2 and revealed that at least four landraces had some level of genomic admixture. This partition primarily differentiated the onions from the Iberian Peninsula from those originating in other European regions.

This preliminary study points to a high degree of genetic diversity within European onion genetic resources and brings to light the necessity of further research, not only for the characterization of genetic variability, but also for the assessment of trait variation and search for promising traits

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Assessing the genetic variation in cultivated tomatoes (*Solanum lycopersicum* L.) using genome-wide single nucleotide polymorphisms

Sung-Chur Sim[a], Myungkwon Kim[b], Sang-Min Chung[c], Younghoon Park[d] and **Youngwhan Choi[d]**

[a]*Department of Bioresources Engineering, Sejong University, Korea*
[b]*Tomato Life Science & Research Center, Chungju, Korea*
[c]*Department of Life Science, Dongguk University-Seoul, Korea*
[d]*Department of Horticultural Bioscience, Pusan National University, Korea*

ywchoi@pusan.ac.kr

Tomato (*Solanum lycopersicum* L.) is an economically important vegetable crop worldwide. Recently, a high-density single nucleotide polymorphism (SNP) array was developed based on genome-wide SNPs in tomato. In this study, we genotyped a collection of 48 Korean elite tomato varieties (26 fresh market and 22 cultivated cherry) using 7,720 SNPs of this array. Out of 6,652 polymorphic SNPs (86.1%) in the entire collection, there were 6,589 SNPs with < 10% missing data. The number of polymorphic SNPs in the fresh market and cultivated cherry sub-populations were 4,733 (61.3%) and 6,087 (78.8%), respectively. In order to examine genetic variation between sub-populations, the SNP genotypes of the Korean tomato germplasm were analyzed along with the previously reported data on SNP of the 277 Solanaceae Agricultural Coordinated Project (SolCAP) varieties (109 fresh market, 27 cultivated cherry, and 141 processing). Principal component analysis, pairwise F_{st}, and Nei's standard genetic distance revealed genetic differentiation between these five sub-populations. Moreover, we validated another division within the Korean cherry varieties using the unweighted pair group mean algorithm (UPGMA). The genetic diversity of each sub-population was estimated based on allelic richness and expected heterozygosity. The fresh market and cultivated cherry sub-populations in the Korean tomato germplasm showed similar levels of genetic diversity as the corresponding SolCAP sub-populations. Visualization of the polymorphic information uncovered genomic regions that differed between the two sub-populations in the Korean tomato germplasm. These results suggest that diversifying selection for market niches and environmental adaptation has led to allelic variation in cultivated tomatoes in Korea.

Diversity of genetic stabilities measures, how to interpret them?

Ridwani Sobir[a], Suprayanti Martia Dewi[b] and Muhammad Syukur[a]

[a] *Department of Agronomy and Horticulture, Bogor Agricultural University, Indonesia*
[b] *Faculty of Agriculture, Mataram University, Indonesia*

rsobir@yahoo.com

Global warming already made fast changed in microclimate for crop production; therefore genotypes with high genetic stability will be key factor to ensure good yield. The challenge is confusion related to diversity of published stability analysis statistic. There were two concepts of stability, static and dynamic stability, where in static stability a stable genotype tends to maintain a constant yield across environments, while in dynamic stability a stable genotype a yield response in each environment that is always parallel to the mean response of the tested genotypes. Based on basic calculation approach we can divide into parametric and non-parametric calculation, in order to elucidate nature of each analysis methods we apply parametric method of Francis and Kannenberg, Wrickle ekovalens, Finlay and Wilkinson, Eberhart and Russel, Wrickle ekovalens and AMMI as well as non-parametric method of Thennarassu and Nassar & Huehn to calculate yield stability of 14 tomato genotypes grown in four lowland locations under Randomized Complete Blocks Design. Based on the concept of static stability using the method of Francis and Kannenberg and Russell Eberhart, the stable genotype was IPBT78, and based on the concept of dynamic stability using Wrickle ekovalens and Finlay & Wilkinson methods, stable genotype was IPBT60, subsequently AMMI method IPBT34 consider to stable genotype. Based on nonparametric stability index values, the stable genotypes in the lowlands by the Thennarassu and Nassar & Huehn methods were IPBT34 and IPBT60, indicated that Thennarassu and Nassar & Huehn methods can be considered represented dynamic stability concept.

Plenary session 4a:
Phenomics I

Phenotyping for plant breeding: combining precision with throughput

Matthew Reynolds, Gemma Molero, Francisco Pinto and Sivakumar Sukumaran

Global Wheat Program, CIMMYT, Mexico

m.reynolds@cgiar.org

Advances plant phenotyping have obvious application in plant breeding and are very timely, considering an increasing global population and the serious challenges to food security associated with climate change. Staple crops are currently showing annual genetic gains of between half and one percent which is insufficient to match predicted global demand. High throughput and precision phenotyping are tools that can be applied directly in breeding to improve the physiology of the crop, as well as in gene discovery, screening of genetic resources, and the genetic and physiological dissection of yield gains.

High throughput generally involves use of non-invasive approaches such as proximal or remote sensing of spectral reflectance from crop canopies. The traits measured relate either to thermal/hydration properties of plant tissue assessed in the infrared region of the electromagnetic spectrum, or pigments related to photosynthesis estimated in visible band. Spectral indices are calculated using different combinations of wavelengths that relate to a range of traits such as canopy temperature, water relations, photosynthesis, nitrogen status, as well as agronomic traits. Ground based proximal sensing of these indices has been available for several decades and can be used to select among genotypes. For example, water index is predictive of cultivar level differences in leaf and soil water potential, as well as agronomic traits (Gutierrez et al., 2010). Normalized difference vegetation index (NDVI) can be used for simple growth analysis.

The most recent advance in the area of high throughput approaches has been the adoption of aerial imaging platforms. These are revolutionizing field phenotyping by offering two main advantages that increase throughput and precision compared to ground-based systems. One is the ability to include hundreds of plots in a single image, reducing the confounding effects of environmental drift associated with time-consuming plot-to-plot measurement. In addition, image analysis also permits data clean-up of pixels (within each plot) that represent 'outliers'. These two factors combined improve associations between remote-sensed traits and yield in comparison to ground based readings. As sensor and image analysis technologies develop the scope and precision of remote sensing is expected to increase.

High throughput phenotyping is ideal for application in QTL analysis which requires measurement of large populations to achieve adequate genetic resolution. Canopy temperature has been used to identify QTL for drought and heat stress tolerance, and QTL common to both stresses were linked to adaptive root response (Pinto and Reynolds, 2015). The same tools are applied in genetic resource screening and have been used to identify valuable new sources of traits associated with abiotic stress adaptation to amplify the breeding genepool (Reynolds et al., 2015).

To accelerate yield improvement, physiological traits (PTs) need to be incorporated into breeding. Physiological breeding applies knowledge of adaptive traits to improve yield potential and stress adaptation. The approach considers a larger range of traits than conventional breeding, including genetically complex physiological characteristics. It also differs from molecular breeding by encompassing both phenomic and genomic information. The main steps are:

1. Designing a plant type with theoretically improved adaptation.
2. Implementing phenotyping protocols and experimental treatments to maximize resolution of physiological trait expression.

Field Phenotyping Platform (FIP) - an automated multi sensor system for plant phenotyping in the field – first results

Norbert Kirchgessner, Frank Liebisch, Kang Yu, Lukas Kronenberg, Andreas Hund, **Achim Walter**

Institute of Agricultural Sciences, Federal Institute of Technology Zürich (ETHZ), Switzerland

Achim.walter@usys.ethz.ch

Plant phenotyping is very important in modern breeding. Genotype evaluations ideally are performed in the target environment on large numbers of replicates and genotypes for critical traits related to yield. We set up a platform enabling the automatic monitoring of multiple plant traits using remote-sensing techniques. A rigged sensor carrier system was installed at ETH Research Station for Plant Sciences Eschikon, Lindau. It allows for free positioning of the sensors in 3D above a field of 1 ha area.
The rigging system including the sensor head and control software was designed and manufactured by Spidercam®. Four 24 m high poles were set up at the four corners of the field with a pulley mounted on top and a winch located in a hut at each base. The pulleys deflect the ropes from the sensor head to the winches. Coordinated adjustment of rope lengths by the winches allows free positioning of the sensor head in three dimensions above the field. Seven sensors were mounted on the head i.e. a visible-, a near infrared, a thermal camera, two spectrometers, a multispectral camera and a terrestrial laser scanner. The sensor unit includes a motorized pan and tilt axis which allow for arbitrary observation directions of the sensors. An integrated operational camera gives a live image of the sensors' perspective for positioning purpose. Considering the maximum payload of 12 kg the sensors can be exchanged keeping the system flexible for future applications and measurements. A high spatial reproducibility is achieved by accurate control of rope lengths and an integrated position calibration of the system.
The complete system of positioning and data acquisition is freely programmable. Therefore measurement scripts were set up for each experiment, enabling the automated acquisition of all plots of an experiment. To date various measurements are performed in automatic, supervised operation. This includes twice a week, plot-wise monitoring of a winter wheat experiment of 700 plots with a near infrared camera which enables robust segmentation and determination of canopy coverage. Moreover, plots of other crops such as soybean, ryegrass and maize were monitored as well. During execution of the measuring script the positions of the plots are successively approached by the sensor head and the sensors measure at each position. Data is automatically stored in the experiment file system and named appropriately. Data examples for several sensors will be presented and related to breeding relevant plant traits. The setup of the system offers an economic, robust and fast data acquisition of large data sets for plant phenotyping in the field. It therefore represents a novel and promising phenotyping platform, complementing the spectrum of existing systems.

Association mapping for transition to autotrophic growth under chilling conditions in maize

Camille Clipet[a], Jean-François Hû[b], Renaud Rincent[c], Sandra Negro[c], Stéphane Nicolas[c], Cyril Bauland[c], Jacques Laborde[e], Delphine Madur[c], Valérie Combes[c], Thomas Altmann[d], Dominique Brunel[f], Marie-Christine Le Paslier [f], Laurence Moreau[c], Alain Charcosset[c] and **Catherine Giauffret[a]**

[a] *UR AgroImpact, INRA, France*
[b] *UE GCIE, INRA, France*
[c] *UMR GQE–Le Moulon, INRA, Université Paris-Sud, CNRS, France*
[d] *Max-Planck Institute for Molecular Plant Physiology, IPK, Germany*
[e] *Unité expérimentale maïs, INRA, France*
[f] *UR EPGV, INRA, Centre National de Génotypage, France*

Catherine.Giauffret@mons.inra.fr

In maize, transition to autotrophic growth is hampered by cold temperatures. Plants from susceptible lines can die after seed reserves have been exhausted if temperatures remain below 13°C. In this study, we used genome-wide association mapping to identify single nucleotide polymorphisms (SNP) related to the ability of plants to transition to autotrophy under chilling conditions. We analysed two highly diverse panels representing two complementary heterotic groups widely used in Northern Europe: 273 dent and 266 flint lines were genotyped with a 50k SNP array and by sequencing, providing approximately 337,000 (dent) and 198,000 (flint) useful markers for genome-wide approaches. The lines were crossed to a tester of the complementary pool and phenotyped as hybrids in cold greenhouses. Each panel was evaluated with 3 replications for several traits (leaf number, leaf size, vigour and colour rating, chlorophyll content, aerial biomass). For the flint panel, we also assessed the quantum yield efficiency of photosystem II. We used spatial models to compute least square means which take into account spatial heterogeneity within each greenhouse. Repeatability on entry means was between 0.67 and 0.88 for the dent panel and between 0.55 and 0.89 for the flint panel. Association mapping yielded 133 and 484 significant SNPs for the dent and flint panels respectively, corresponding to 77 and 378 QTLs. The SNPs explained up to 19% of the phenotypic variance. All QTLs detected for the flint panel were related to the quantum yield efficiency of photosystem II, suggesting that this trait is highly valuable for screening genetic variation in flint material during transition to autotrophic growth under cold conditions.

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Imaging of maize root traits in multiple field environments reveals high heritability but limited genotype-specific response to low nitrogen

Chantal Le Marié[a], Karl-Heinz Camp[b], Alexandre Strigens[b], Jonathan P Lynch[c], Larry York[cd], Silvio Salvi[e], Marcos Malosetti[f] and Andreas Hund[a]

[a] *Institute of Agricultural Sciences, ETH Zurich, Switzerland*
[b] *Delley seeds and plants Ltd, Switzerland*
[c] *Department of Plant Science, The Pennsylvania State University, United States*
[d] *Centre for Plant Integrative Biology, University of Nottingham, United Kingdom*
[e] *Department of Agricultural Sciences, University of Bologna, Italy*
[f] *Department of Plant Science, Wageningen University, The Netherlands*

Chantal.lemarie@usys.ethz.ch

Due to the need for sustainable intensification of agriculture in the coming decades, excessive nitrogen (N) fertilization is not justifiable from ecological and economic perspectives. One opportunity to reduce N application rates without major losses in yield is breeding for nutrient efficient crops. One key parameter that influences nutrient uptake efficiency is the root system architecture (RSA). To explore the impact of N availability on RSA and to investigate the impact of the growth environment, a diverse set of 36 inbred dent maize lines crossed to the inbred flint line UH007 as a tester was evaluated for N-response over two years in three environments. RSA was investigated by excavating and imaging the root crowns followed by image analysis with REST software. Despite strong site and year effects, trait heritability was generally high. Root traits showing the highest heritability (> 0.7) were the width of the root system, indicative of the horizontal expansion, and the fill factor, a measure of the density of the root system. Heritabilities were in a similar range under high or low N application. Under N deficiency the root system size decreased, the horizontal expansion decreased and the root system became less dense. However, there was little differential response of the genotypes to low N availability. Thus, the assessed root traits were more constitutively expressed rather than showing specific genotypic adaptations to low N. In contrast, strong differences were observed for 'stay green' and silage yield, indicating that these highly heritable traits are good indicators for responsiveness to low N.

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Ideotype based breeding for sugarcane yield: a case study of using logistic regression models to identify optimum trait combinations in different breeding populations.

Marvellous Zhou[a,b]

[a] *Breeding and Field Resource Unit, South African Sugarcane Research Institute, South Africa*
[b]*Department of Plant Breeding, University of the Free State, South Africa*

Marvellous.Zhou@sugar.org.za

Ideotype breeding aims to produce cultivars that possess optimum trait combinations leading to high productivity. In sugarcane, cane yield is controlled by number, height and diameter of stalks. The objectives of this study were to identify the optimum combination of number, height and diameter of stalks that produce high cane yield in different sugarcane breeding populations and determine the magnitude of yield increase when the optimum trait combinations were applied during selection. Data for number, height, diameter of stalks and cane yield were measured from seven breeding populations, each representing an agro-ecological region in the South African sugarcane growing belt. Of the seven breeding populations, two were derived from the high altitude Midlands region breeding programmes representing humic and sandy soils, one represented the irrigated region and four represented the coastal regions. Sugarcane is grown under rainfed conditions in the Midlands and coastal regions. The Midlands crop is harvested at 24 months crop age, the irrigated at 12 months while the coastal programmes are harvested at 12-18 months. Each population was made up of more than 2,000 genotypes and each genotype was planted to a single row of 8 metres long. Thirty percent of the data were used to produce logistic regression models that determined the optimum combination of number, height and diameter of stalks for predicting cane yield while 70% of the data were used to test the ability of the models to select genotypes that produced high cane yield. The predictor variables (number, height and diameter) produced significant ($P<0.01$) chi-square values indicating strong influence on predicting cane yield of a genotype. The order of importance of number, height and diameter of stalks was ranked based on chi-square values. For the Midlands populations, number and diameter of stalks were equally more important than stalk height. For the coastal 18 month populations, number of stalks was the most important trait followed by height for the coastal average potential population and diameter for the coastal hinterland population. For the coastal 12 month populations, diameter was most important followed by number of stalks. In the irrigated populations, diameter was more important than number of stalks while height was by far the least important. The results suggest that for 12 month crop cycles, diameter of stalks is more dominant in predicting cane yield while on long cycle crops (18-24 months), number of stalks were more important. The models selected genotypes that produced significantly ($P<0.0001$) higher cane yield than the control cultivars. The selected genotypes produced, on average, 43% to 89% more cane than the low yielding genotypes and 29% to 141% more cane than the commercial cultivars used as controls in the trials. The genotypes identified as high yielding produced consistently higher numbers of stalks that were taller and thicker than the low yielding genotypes across all the breeding populations. The results suggest that the agro-ecological regions require different combinations of number, height and diameter of stalks to achieve high cane yield. Using the correct combination of number, height and diameter of stalks during selection would identify the higher yield genotypes, further accelerating genetic gains. Logistic regression models would increase objectivity in identifying genotypes that combine the optimum number, height and diameter of stalks, further increasing efficiency of selecting high cane yield genotypes in sugarcane breeding.

Plenary session 4b:
Phenomics II

Combining phenotyping, genetic analyses and crop modelling for identifying traits and alleles of tolerance

Francois Tardieu[a], Emilie Millet[a], Santiago Alvarez Prado[a], Aude Coupel-Ledru[a], Tsu Wei Chen[a], Llorenc Cabrera Bosquet[a], Sebastien Lacube[a], Margot Leclère[a], Christian Fournier[a], Boris Parent[a] and Claude Welcker[a]

[a] *INRA LEPSE, Montpellier, France*

francois.tardieu@supagro.inra.fr

The plant science community has to designing new genotypes able to cope with climate changes. Phenotyping is the main limiting step, now that genotyping has become an almost routine activity. Many platforms in the field and in controlled conditions are now available, but methods and pipe lines of analysis are now required to transform raw data into precise, useful and relevant knowledge on how a large range of genotypes respond to their environment, and what is the genetic basis for variability of responses. We base our approach on a combination of methods involving genetic analyses of traits in phenotyping platforms and of performance in the field, together with the use of crop modelling. The rationale is that a given trait or allele confers advantages for yield in specific scenarios of water deficit or high temperature, but most often not in all drought scenarios. It is therefore necessary (i) to explore, in networks of field experiments, a series of environmental scenarios for identifying in which scenarios a given allele has positive, negative or no effect on yield, (ii) to dissect these effects into responses to specific environmental conditions and their genetic variability, (iii) to simulate via a crop model the effects of combinations of alleles in a large range of environmental conditions. I shall present the results of this strategy, applied to a panel of 250 maize hybrids allowing a multi-scale multi environment whole-genome association study

1. We have developed methods for investigating the genetic variability of responses to light, evaporative demand and soil water deficit.

- Light interception and radiation use efficiency are essential components of biomass accumulation and of its change with environmental conditions. Genetic analyses require thousands of plants, so phenotyping is nearly impossible to carry out with gas exchange measurements. Phenotyping platforms able to characterize both plant architecture and biomass accumulation can provide new insights. We have developed a suite of methods able to evaluate the amount of light intercepted by plants, via a functional-structural model using 3D reconstructions of each plant placed in a virtual scene reproducing the canopy in the greenhouse (RATP model). This needs prior evaluation of the spatial distribution of incident light as experienced by hundreds of plants in a greenhouse, by simulating sun beam trajectories through greenhouse structures every day of the year. Combined with a precise analysis of the spatial and temporal analyses of environmental conditions, this method has allowed us to analyse the genetic variability of a proxy of photosynthesis, radiation use efficiency, and of light interception as a function of plant architecture.
- The sensitivity of plant growth to water deficit can be evaluated in a platform via joint analysis of several experiments, via response curves of leaf expansion rate to soil water potential. We have performed this analysis in four experiments with contrasting environmental conditions. A whole-genome association study reveals a clear genetic architecture, with interesting co-location of sensitivity with QTLs of transcripts of genes involved in hydraulic processes.

2. In the field, we have adopted a multi-environment approach that consists of (i) clustering time courses of environmental variables simulated by a crop model in current and future conditions (35 years × 55 sites), into six scenarios of temperature and water deficit as

Enhancement of SAT agricultural production; Development of trait-based environment specific breeding pipeline @ ICRISAT

Vincent Vadez[a], **Jana Kholová** [a], Graeme L. Hammer[b] and Michael Blummel[c]

[a] *International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India,*
[b] *The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Australia*
[c] *International Livestock Research Institute, Patancheru, India*

j.kholova@cgiar.org

Enhancement of agricultural production requires syntheny of disciplines addressing the major constrains of target systems. Semi-arid cropping systems are usually water-limited which predetermines frequent occurrence of terminal droughts. In such conditions, any crop adaptation (e.g. water use dynamics or access to water) which can enhance water availability during the grain filling also improves crop production. We identified a range of plant mechanisms altering the crop water use during the season and translating into production advantage under prevalent environments in target systems.

To project the knowledge of plant adaptations into application within the breeding program this has to be coupled with relevant upstream and downstream research. From one hand, it is essential to understand the genetic determination of any trait of interest. From the other hand, accurate and rapid assessment of desired phenotype is necessary to enable selection within the breeding populations. Ultimately, the magnitude of production benefits/trade-offs under-lied by particular trait has to be demonstrated within the target agro-eco-systems to justify any resource investment involved in traits-based breeding approaches.

At ICRISAT, we have developed pipeline of interdisciplinary capacities/tools/techniques to justify and employ the trait based breeding pipeline for crops production improvement at SAT agro-ecologies. The current status of pipeline development will be the focus of the presentation; i) the core knowledge of drought adaptations (e.g. canopy development, structure, conductivity), ii) suitable technology enabling HT-phenotyping for particular drought adaptations iii) linkage of drought adaptations mechanisms to crop production (phenotyping pipeline outputs up-to-date), iv) knowledge of adaptive traits genetics (QTL co-localization across phenotyping systems), v) use of crop models to predict the trait value for particular ExM context vi) linkage to socio-economics of the region.

Modelling the growth of perennial ryegrass under water limiting conditions

Kristina Jonavičienė[a,b], Steven Yates[b], Sebastian Nagelmüller[b], Frank Liebisch[b], Norbert Kirchgessner[b], Achim Walter[b], Gintaras Brazauskas[a] and Bruno Studer[b]

[a] *Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Lithuania*
[b] *Institute of Agricultural Sciences, ETH Zürich, Switzerland*

kristinaja@lzi.lt

Water limitation, caused by drought, salinity and frost, is one of the major abiotic stresses limiting plant growth and crop productivity worldwide. Maintaining high and stable yields under unfavourable conditions is a priority for crop improvement by breeding. However, direct selection for yield under water limiting conditions has proven to be difficult, due to its dynamic nature and the difficulty to quantitatively associate growth with water stress.
Here, we present a novel, non-destructive and largely automated phenotyping platform for the real time analysis of the leaf elongation rate (LER) in perennial ryegrass (*Lolium perenne* L.) under water limiting conditions. The data obtained were integrated into an ecophysiological model to investigate the response of the LER to temperature and soil moisture. The model is developed for use in a dynamic environment and takes into account environmental variables to describe leaf elongation in grasses. According to the model, plant growth in response to water deficit is not linear but has three phases demarcated by growth reduction and growth arrest. The first phase depicts "normal" growth, when water in soil is freely available and the growth is mainly governed by temperature, followed by the second phase "decrease", which is attributable to temperature and soil moisture and the terminal phase "arrest", where leaf growth has stopped. The results were highly reproducible and revealed large differences in a diverse panel of perennial ryegrass genotypes.
The ability to decompose complex data into quantitative parameters and to pinpoint growth reduction and arrest allows for direct selection of these traits in breeding programs and further enables association analysis in genetically characterized populations leading to identification of QTLs underpinning the phenotype. The versatility of the method enables its adaptation for studying other abiotic (salinity, osmotic, heavy metal) and chemical (fertilizer, pesticide) stresses of perennial ryegrass and other graminoid species. Therefore, the method described here, has a strong application for both fundamental and applied research to improve crop productivity.

Whole Genome QTL Search for Root System Architecture in Tetraploid Wheat

Marco Maccaferri[a], Walid El-Feki[b], Nazemi Ghasemali[c], Chiara Colalongo[a], Danara Ormanbekova[a], Silvio Salvi[a], Sandra Stefanelli[a] and Roberto Tuberosa[a]

[a] *DipSA - University of Bologna, Bologna, Italy,*
[b] *Department of Crop Sciences, Faculty of Agriculture, Alexandria University, Alexandria, Egypt,*
[c] *Department of Agriculture, Haji Abad Branch, Islamic Azad University, Haji Abad, Iran*

marco.maccaferri@unibo.it

Optimization of root system architecture (RSA) traits is an important objective for modern wheat breeding. Two recombinant inbred line populations and one association mapping panel of 183 elite durum wheat (*Triticum turgidum* L. var. *durum* Desf.) accessions evaluated as seedlings grown on filter paper/polycarbonate screening plates revealed 20 clusters of quantitative trait loci (QTLs) for root length and number, as well as 30 QTLs for root growth angle (RGA). Divergent RGA phenotypes observed by seminal root screening were validated by root phenotyping of field-grown adult plants. The seminal root survey was extended to a collection of 200 *T. dicoccum* and 150 *T. dicoccoides*. Among RSA, RGA appeared as one of the traits most affected by domestication process. QTLs were mapped on a high-density tetraploid consensus map based on transcript-associated Illumina 90K single nucleotide polymorphisms (SNPs) developed for bread and durum wheat, thus allowing for an accurate cross-referencing of RSA QTLs between durum and bread wheat. Among the main QTL clusters for root length and number highlighted in this study, 15 overlapped with QTLs for multiple RSA traits reported in bread wheat, while out of 30 QTLs for RGA, only six showed co-location with previously reported QTLs in wheat. Based on their relative additive effects/significance, allelic distribution in the association mapping panel, and co-location with QTLs for grain weight and grain yield, the RSA QTLs have been prioritized in terms of breeding value. Based on the most recent wheat genome assemblies, the gene content of the RGA QTLs is being considered for candidate gene analysis. Three major QTL clusters for root length and number (RSA_QTL_cluster_5#, RSA_QTL_cluster_6#, and RSA_QTL_cluster_12#) and nine RGA QTL clusters (QRGA.ubo-2A.1, QRGA.ubo-2A.3, QRGA.ubo-2B.2/2B.3, QRGA.ubo-4B.4, QRGA.ubo-6A.1, QRGA.ubo-6A.2, QRGA.ubo-7A.1, QRGA.ubo-7A.2, and QRGA.ubo-7B) appear particularly valuable for further characterization towards a possible implementation of breeding applications in marker-assisted selection and/or cloning of the causal genes underlying the QTLs.

Genetics and genomics of water stress tolerance in wild tomato

Dina A. St.Clair, Erin M. Arms, Jared Lounsbery, Arnold J. Bloom

Plant Sciences Department, University of California Davis, United States of America

dastclair@ucdavis.edu

Global climate change and limited fresh water resources pose significant challenges to agricultural production systems worldwide. Breeding crops for tolerance to water stress and increased water use efficiency (WUE) would improve the sustainability of crop production by requiring less water for a given level of yield. Cultivated tomato (*Solanum lycopersicum*) is susceptible to many abiotic stresses, including limited water and chilling temperatures. A wild tomato species (*S. habrochaites*) originating from the Peruvian Andes exhibits tolerance to several abiotic stresses, including limited water and chilling temperatures. Water stress can be rapid-onset or slow-onset. Rapid-onset water stress is induced by root chilling (6°C), reducing water movement from roots to shoots. *S. habrochaites* responds to root chilling by closing stomata and maintaining shoot turgor, while cultivated tomato (*S. lycopersicum*) fails to close stomata and wilts. This trait (shoot turgor maintenance under root chilling) is controlled by a major QTL (*stm9*) on chromosome 9. We high-resolution mapped *stm9* to a 0.32 cM region using a set of sub-near-isogenic lines (sub-NILs) for chromosome 9 from *S. habrochaites*. To determine if other traits associated with water stress tolerance mapped to this region, we evaluated this same set of sub-NILs in replicated field experiments for two years under severely restricted irrigation (i.e., slow-onset water stress). Traits measured included fruit yield, shoot dry weight (biomass accumulation), specific leaf area, and mature leaf delta-13C, a measure of carbon isotope discrimination (13C:12C). Delta-13C is correlated with WUE. Trait data obtained for the sub-NILs was used to map QTL, determine QTL linkage relationships and QTL x Environment interactions. The majority of the QTL for the measured traits were closely linked to, but not coincident with, *stm9*. Almost all traits evaluated in the field experiments segregated in the sub-NILs derived from *S. habrochaites*, but some QTL positions were not fully resolved. Therefore, we generated a new set of sub-NILs containing *S. habrochaites* chromosome 9 introgressions that extend farther towards the centromere. These sub-NILs will be evaluated in field experiments under severely restricted irrigation (slow-onset water stress) and phenotyped for the same set of traits to resolve QTL locations and effects. In the cultivated tomato (*S. lycopersicum*) reference genome, this region of chromosome 9 region is gene-rich, but a reference genome for *S. habrochaites* is not available. Therefore, genomic BACs for this region are being sequenced to identify QTL candidate genes and enable structural genomic comparisons to the cultivated tomato genome. We are also using mRNA-Seq to analyze transcriptional regulation of plant responses to water stress. Collectively, our data suggests that genetic elements on chromosome 9 of *S. habrochaites* play important roles in plant responses to water stress and chilling temperatures. Our research will aid crop improvement efforts and help address challenges imposed by limited fresh-water resources.

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Plenary session 5a:
Genetic resources (conservation)

Crops and culture: conserving the seed heritage

Ola Westengen[a], Kristoffer Ring[a] and Quetzalcoatl Orozco-Ramirez[c]

[a] *Noragric, Department of International Environment and Development Studies, Norwegian University of Life Sciences, Norway*
[b] *Centre for Environment and Development, University of Oslo, Norway*
[c] *Center for Research in Environmental Geography, National Autonomous University of Mexico, Mexico*

ola.westengen@nmbu.no

Crop diversity embodies ten thousand years of agricultural history. The very term plant genetic resources for food and agriculture (PGRFA) connotes that they are *resources* that we conserve for their utilitarian value, but as pioneers in the plant genetic resources movement pointed out, genetic resources is also mankind's *cultural* heritage (Harlan 1975). Implicit in this notion of crop diversity as cultural heritage is a recognition of the fact that not only environmental factors have shaped the genetic diversity in our crops as they spread around the world, but also cultural factors. In this paper we explicitly focus on how cultural factors have shaped geographical patterns in genetic diversity in sorghum and maize. We build on earlier work presenting molecular evidence of close associations between sorghum population structure and the distribution of ethnolinguistic groups in Africa (Westengen et al. 2014) and explore hypotheses of relationships between ethnolinguistic groups and patterns of maize diversity in Mesoamerica. The data for our analyses of maize genetic structure and adaptations is the Seeds of Discovery genome-wide Genotyping by Sequencing (GBS) data for thousands of landrace accessions conserved in the CIMMYT genebank (Hearne et al. 2014). Our spatially explicit analyses reveal evidence of roles of both environmental and cultural factors in shaping genomic diversity at the landscape level. We relate our work to the interdisciplinary farming language dispersal hypothesis which proposes that farming and language families have spread together through population growth and migration (Diamond and Bellwood 2003). The common way to test this hypothesis has been through tests for associations between human genetic structure and language distribution, thus our crop genetic approach complements other disciplinary strands of inquiry into historical and contemporary relationships between crops and cultures. Understanding the relationship between cultural factors and crop diversity has practical value for PGRFA conservation and use in at least two ways: (1) If cultural factors have shaped diversity this warrants complementary ways of targeting germplasm collecting for ex situ conservation priorities as well as for prioritising in situ/on farm conservation: (2) Cultural factors should be taken into account in plant breeding programs for traditional crop homelands. We argue that efforts to strengthen seed systems are more likely to be successful when building on, rather than seeking to replace, existing traditional seed systems and landraces.

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Conservation and exploitation of plant genetic resources – the view of a genebank manager

Andreas Börner[a], Manuela Nagel[a], Mian Abdur Rehman Arif[a,b], Mai Allam[a,c], Monika Agacka-Mołdoch[a,d] and Ulrike Lohwasser[a]

[a] *Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany*
[b] *Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan*
[c] *National Research Center, Cairo, Egypt*
[d] *Institute of Soil Science and Plant Cultivation, State Research Institute, Puławy, Poland*

boerner@ipk-gatersleben.de

Plant genetic resources play a major role for global food security. The most significant and widespread mean of preserving plant genetic resources is *ex situ* conservation. World-wide 7.4 million accessions are stored in about 1,750 *ex situ* genebanks. The largest numbers of accessions stored are of wheat (855,000), rice (775,000), barley (465,000) and maize (325,000). Other large germplasm holdings include bean, sorghum, soybean, oat, groundnut and cotton. One of the ten largest *ex situ* collections of our globe is located at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany, conserving 150,000 accessions from 3,200 plant species and 780 genera. Since the majority of genebank holdings globally are stored as seed, seed storability is of exceptional importance for germplasm conservation. Seed storage is managed in large cold chambers at -18°C. Seeds are kept in glass jars, covered with bags containing silica gel. The maintenance of the collection requires regeneration. Each year between 8 and 10% of the collection is grown either in the field or in glasshouses. Regeneration is carried out locally to ensure genetic integrity and to minimize genetic erosion. Special attention has to be given to out-pollinating species, which are either multiplied in small glasshouses or in isolation plots in the field.

A pre-requisite for any further exploitation activities is to maintain a high seed quality during storage. The lifespan, or longevity, of seeds is crop specific. However, there are also strong hints of an intraspecific variability which is genetically determined. Consequentially, studies were initiated determining genetic loci responsible for seed storability. At IPK investigations were performed on barley, wheat, oilseed rape and tobacco employing both bi-parental mapping populations and association mapping panels.

An efficient exploitation of the germplasm collections is often hampered by the huge numbers of accessions stored in the seedbanks. Therefore, core collections representing the genetic variation of the whole set must be created. The utilisation of such an approach for unlocking the genetic diversity ‘sleeping’ in genebanks will be discussed.

Bottlenecks in the PGRFA sustainable use system: stakeholders' perspectives

Shelagh Kell[a], Mario Marino[b] and Nigel Maxted[a]
[a] *School of Biosciences, University of Birmingham, United Kingdom*
[b] *International Treaty on Plant Genetic Resources for Food and Agriculture, Rome, Italy*

s.kell@bham.ac.uk

The Governing Body of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA – the Treaty) has recognized the pivotal role of sustainable use of PGRFA in addressing global challenges, including biodiversity loss, climate change adaptation, poverty alleviation, and food security—especially for smallholder and subsistence farmers. However, in many regions, the implementation of Article 6, 'Sustainable Use of Plant Genetic Resources' is lagging behind in comparison with other elements of the Treaty. The Governing Body, recognizing the need for further financial resources, capacity building and technology transfer, has Article 6 as a standing priority item on its agenda and has called for the development of a toolbox to assist Contracting Parties in designing measures to promote the sustainable use of PGRFA. An online consultation to gather the views and needs of stakeholders was conducted from April to June 2015 in which all FAO sub-regions were represented across 109 countries and the European Union, of which 90 are Contracting Parties to the Treaty. The results of the consultation have allowed a clearer understanding of the 'bottlenecks' in the sustainable use system and a deeper comprehension of the constraints and needs regarding the implementation of the sustainable use provisions of the Treaty. In particular, there is a critical need to address limitations regarding policy in support of sustainable use activities, as well as capacity building needs in all areas of the PGRFA sustainable use spectrum. Further, access to plant genetic material and associated information urgently needs to be addressed in order that countries can move ahead with the development of coordinated and comprehensive sustainable use strategies.

Genetic diversity of Austrian flax accessions – a case study for *ex situ* germplasm characterisation

Eva-Maria Halbauer[a], Valentina Bohinec[a], Melanie Wittenberger[a], Karin Hansel-Hohl[a] and **Eva M. Sehr**[a]

[a] *Health & Environment Dept., AIT Austrian Institute of Technology GmbH, Austria*

eva-maria.sehr@ait.ac.at

Biodiversity is currently experiencing severe genetic erosion due to mankind's unsustainable activities. This exponential loss of plant genetic diversity through the world has led to initiatives to conserve biological diversity incorporating both *ex situ* and *in situ* techniques. Particular the former, the conservation of components of biological diversity outside – *ex situ* – their natural habitats, is an important strategy for crops, their landraces and wild relatives, since very often original habitats are under threat. So far, approximately seven million crop accessions are currently being conserved in collections worldwide illuminating the multiplicity of adaptive genetic responses that plant populations have evolved to cope with environmental and physiological stress. In order to unlock the wealth of diversity that exists in genebanks, the characterization of this material via the application of state-of-the-art genomic, phenomic and molecular technologies is required. This is a very critical part of any long-term strategy to enhance the productivity, sustainability and resilience of crop varieties and agricultural systems.

In this study, we characterised the genetic diversity of flax (*Linum usitatissimum* L.), a well-known crop species used for its fibres and oil, by using 24 Austrian and 4 Swiss accessions provided by 5 different genebanks / sources. Twenty nuclear microsatellite markers (nSSRs) were chosen from literature [1] and tested for their usability. Out of these, 11 markers were applied on a core sample set comprising 6 accessions revealing mean gene diversity values (He, D) of 0.102 to 0.451 per accession. Five of the most polymorphic markers were further used on the total set of 28 accessions. Besides the characterisation of the accession-specific genetic diversity we uncovered an intermixture of accessions. That could be explained by the fact that these accessions were coming from the same seed provider.

Such an in-depth genetic analysis of accessions stored *ex situ* not only produces valuable agronomic and breeding data, but also is useful for the identification of duplicates within and between collections, contributing to the potential for rationalisation of collections, which in turn can help ensure that the limited resources available for regeneration are used most efficiently and effectively.

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Assessing global patterns of genetic diversity and population structure of tetraploid gene pool of alfalfa (lucerne)

Doğan İlhan [a], Xuehui Li [b], E. Charles Brummer [c] and **Muhammet Şakiroğlu [d]**

[a] *Molecular Biology and Genetics Department, Kafkas University, Kars, Turkey*
[b] *Department of Plant Sciences, North Dakota State University, USA*
[c] *Department of Plant Sciences, The University of California-Davis, USA*
[d] *Department of Bioengineering, Kafkas University, Kars, Turkey*

msakiroglu@kafkas.edu.tr

The *Medicago sativa-falcata* complex includes the economically important forage legume alfalfa and its primary gene pool. The complex includes both diploid and tetraploid taxa, which are usually designated as distinct subspecies. The relationships among wild diploid members of the complex have been clarified using molecular markers, but the relationships among unimproved tetraploid germplasm is poorly understood. Our aim was to investigate the population genetic structure of the tetraploid *Medicago sativa-falcata* complex to deduce the amount and pattern of genetic diversity using genome-wide SSR markers. We used 70 tetraploid accessions (280 genotypes) from the USDA National Plant Germplasm Collection that were collected from throughout the entire natural distribution range of the species and that represented putative wild populations. Population genetic analyses were conducted to determine the patterns of demarcation among accessions and germplasm groups. Model-based cluster analysis indicated that tetraploid alfalfa has two main groups corresponding to the subspecies *sativa* and *falcata*. *Medicago sativa* subsp. ×*varia* produced a hybrid pattern in between *Medicago sativa* subsp. *sativa* and *Medicago sativa* subsp. *falcata*. The studies also revealed that there is a spatial genetic structure among subsp. *falcata* accessions, implying that extensive sampling from different localities for curation of alfalfa genetic resources is important. Because cultivated alfalfa is largely tetraploid, the results of this experiment can help guide the introduction of new tetraploid germplasm into the breeding programs.

Plenary session 5b:
Genetic resources (pre-breeding)

Multi-parent populations for dissection of complex traits in rice

Hei Leung, Chitra Raghavan, Monalisa Jubay, Justine Bonifacio, Arnel Pocsedio, Hein Zaw, and Rakesh K. Singh

International Rice Research Institute, Los Banos, Laguna, Philippines

h.leung@irri.org

MAGIC *(***M**ulti-parent **A**dvanced **G**eneration **I**nter-**C**rosses*)* is a breeding method that increases the precision in mapping quantitative trait loci (QTL) for multiple traits. The highly recombined populations can be used directly as source materials for the extraction and development of breeding lines and varieties adapted to different environments. The increased recombination in MAGIC populations can lead to novel rearrangements of alleles and greater genotypic diversity (Huang et al 2015).

Using elite lines as founders, we have developed 4 multi-parent populations: *indica* MAGIC (8 *indica* parents); MAGIC plus (8 *indica* parents with two additional rounds of 8-way F1 inter-crossing); *japonica* MAGIC (8 *japonica* parents); and Global MAGIC (16 parents – 8 *indica* and 8 *japonica*) (Bandillo et al 2013). All the 16 parents are improved varieties with desirable traits for biotic and abiotic stress tolerance, yield, and grain quality. The lines were cycled through multiple generations of out-crossing. Each generation of random mating reduces the extent of linkage disequilibrium (LD), allowing the QTL to be mapped accurately. Lines derived from early generations can be used for QTL detection and coarse mapping while those derived from later generations will detect marker-trait associations. This allows fine mapping of multiple traits on the same population.

We estimated the number of observable recombinatons in the indica MAGIC population (1316 lines) across varying levels of SNP markers. We observed that the number of recombinations increased as markers increased, but with an increase in markers levels, genotyping errors also incresed. We also noted that it was critical to bin the markers (i.e. spacing the markers in intervals) to avoid false calls of recombination. Filtering criteria applied to GBS data also affects the estimates. In the indica MAGIC population we estimated approximately 98 recombinations per advanced inbred line (AIL) using 8,445 markers spaced at 0.1cM and at 0.03 genotying error probability. The threshold for estimating founder probability was set to 0.7.

Traits for abiotic, biotic stress tolerance, grain quality and agronomic traits have been mapped in the indica MAGIC population. Using 6,166 markers (binned at 0.1cM) we were able to map QTLs to less than 1 cM intervals (average of 250 kb) for the traits examined so far. Using the yield data from two dry seasons, two significant QTLs for yield were identified on chromosomes 3 and 8 with a percent variance of greater than 2 by both GWAS and interval mapping approaches. The QTL on chromosome 3 colocalizes with a previously identified QTL for heading date. We also mapped days to flowering and note that it colocalizes with the yield QTL on chromosome 3. The QTL detected on chromosome 8 colocalizes with a QTL reported for filled grain weight previous described in an analysis of source-sink activity. A third QTL on chromosome 4 was also detected using interval mapping approach at $p < 0.001$ accounting for 1.36% phenotypic variance. The QTL on chromosome 4 aslo colocalizes with a QTL for source activity and a seed related QTL (http://qtaro.abr.affrc.go.jp/cgi-bin/gbrowse/Oryza_sativa/#search).

Yield phenotype is a complex representation of QTL interactions. To understand the interactions among the multiple traits we used the Bayesian Network package R/bnlearn to examine the interactions among 12 traits, which include biotic, abiotic stress, grain quality and agronomic traits affecting yield in the indica MAGIC population. Preliminary results

Molecular diversity of fungal disease resistance genes in cereals and their applications in breeding

Beat Keller[a], Severine Hurni[a], Kaitlin E. McNally[a], Simon G. Krattinger[a], and Salim Bourras[a]

[a] *Department of Plant and Microbial Biology, University of Zurich, Switzerland*

bkeller@botinst.uzh.ch

The cereal genomes are large and highly complex. Nevertheless, in the last years genomic resources have been developed which allow a more efficient characterization of genetic diversity and also the molecular identification of agronomically important genes. Gene isolation, allele-mining and whole genome-based analyses have all contributed to a better characterization of cereal diversity at the molecular level. While characterizing genetic diversity is very important, there is also a need to ensure the application of the obtained knowledge in cereal breeding. We are using the newly developed genomic approaches for the study of the origin, evolution and functional diversity of R genes in the wheat gene pool. Based on the existing natural diversity, we are exploring ways to make artificial diversity by designing novel, synthetic R genes with broader functional spectrum and improved durability.

R genes frequently encode NB-LRR (NLR) immune receptors that confer race-specific resistance against pathogens. This type of resistance is frequently overcome by newly evolved pathogen races. The use of different genes in multi-lines or the combination of different resistance genes or alleles in F1 or in pyramided lines is proposed as a strategy for more durable resistance. We found that the combination of different alleles of the powdery-mildew-resistance gene *Pm3* in F1 hybrid and double-transgenic wheat lines can result, in some cases, in a suppression activity among the *Pm3* alleles. We also studied the suppression in wheat of the powdery mildew resistance gene *Pm8* originally introgressed from rye. Some wheat lines with a partial translocation of chromosome 1R do not show resistance to isolates of the wheat powdery mildew pathogen avirulent to *Pm8*. We found that the wheat gene *Pm3*, an ortholog of rye *Pm8*, suppressed *Pm8*-based resistance in a transient expression assay. This result was further confirmed in transgenic lines where both *Pm8* and *Pm3* transgenes were present. This suggests that the expression of closely related NLR resistance genes in the same genotype can lead to dominant-negative interactions, which is a molecular explanation for and a first step to overcome the frequently observed ineffectiveness of resistance genes introduced from the secondary gene pool into breeding lines.

In addition to the NLR genes described above, quantitatively acting resistance genes are of great relevance for breeding and research. Such genes frequently act additively and result in agronomically useful, durable resistance, but very few of them have been identified at the molecular level. We are specifically characterizing molecularly QTL conferring leaf rust resistance in wheat and leaf blight in maize. The wheat rust QTL Lr34, encodes a putative ABC transporter protein and we are studying its function at the molecular level. Interestingly, the Lr34 gene can be transferred into other cereal crop species. In particular, we have transformed the gene into barley and rice where it confers the characteristic properties of Lr34: leaf tip necrosis and resistance to biotrophic or hemi-biotrophic pathogens. The maize Htn1 gene encodes a receptor-like protein, further expanding the repertoire of quantitatively acting genes in cereals.

QTL identification and phenotyping of strawberry fruit of an octoploid population for quality traits

Rashed Alarfaj[a], Richard Harrison[b] and Carol Wagstaff[a]

[a] *Department of Food and Nutritional Sciences, University of Reading, UK*
[b] *Genetics & Crop Improvement programme, East Malling Research (EMR), UK*

R.A.R.Alarfaj@pgr.reading.ac.uk

Strawberries are very perishable fruits, so improving their nutritional and quality traits is a fundamental goal for breeding programmes. Due to the complexity of the octoploid genome, it is only recently that markers have been developed for breeding purposes. This project aims is to characterise the variation of shelf life and nutritional quality traits among the progeny of the Redgauntlet x Hapil population (RGxH) and therefore to identify Quantitative Traits Loci (QTL). This will set the stage for the improvement of postharvest quality of commercial strawberry varieties based on their genetic potential.

Key fruit quality traits including fresh weight, total soluble solids (TSS), titratable acidity (TA), firmness, phenolic content and colour at different post-harvest days (day 1 and 7 for year 1 and day 1, day 4 and 7 for year 2) were characterised in an octoploid strawberry mapping population, consisting of 188 F1 individuals plus the parents. Traits were phenotyped over two seasons in two different locations to enable the analysis of gene x environment interactions. The existing SNP-based linkage map for the population, distributed on 28 linkage groups, was used to identify QTL associated with phenotype data.

From experiments over two sequential years (2013 and 2014), 51 post-harvest traits of the strawberry mapping population were phenotyped. F1 lines showed a large range of variation across the population for each trait and transgressive segregation was observed. 9 and 37 QTLs, distributed on 20 linkage groups, were detected for "year 1" and "year 2" respectively, including some 'hotspot' regions where QTL for multiple traits were co-located. The identification of QTLs for the quality traits of strawberry will lead to a better understanding of the correlations between different traits at the genetic level. Refinement of the QTL in future work will be important for breeding purposes in terms of identifying genetic markers for marker-assisted selection (MAS) programmes, which enable the pre-selection of seedlings process at a very early stage of breeding, thus improving efficiency and enabling a targeted approach.

Supervised genetic group classification for plant breeding

Antoine Botrel[a], Catherine Guidat[b], Emmanuelle Dyrszka[c], Joel Piquemal[c], Nicolas Ranc[d], Mercedes Murua[e], Odile Argillier[c], Pierre Pin[c], Denis Lespinasse[c], Florence Louis[a], Monica Menz[a] and Joe Frinzl[f]

[a] *Genetics Department, Syngenta, France*
[b] *Vegetables R&D, Syngenta, France*
[c] *Diverse Field Crops/Cereals R&D, Syngenta, France*
[d] *Maize R&D, Syngenta, France*
[e] *Diverse Fields Crops/Cereals R&D, Syngenta, USA*
[f] *R&D Information System, Syngenta,USA*

antoine.botrel@syngenta.com

Grouping materials according to genetic similarity is a common breeding practice. The genetic group segmentation helps the breeders to create and validate generalization hypothesis, i.e. check if what is true for a specific material is true for similar materials in the same group. It creates an abstraction level over the genetic information that is necessary for organizing the germplasm and defining the breeding strategy (specialization of the genetic groups in order to gain combining ability and to target different traits).
In this area, most of the current efforts are focused on genetic group identification thanks to clustering methods (unsupervised classification). The clustering task takes as input a genotype matrix and provides an assignment of the individuals in groups based on threshold parameters. The group definition is learnt from the data and might change from one dataset to another. This makes the clustering results unsteady and not sufficient to be interpreted and used by breeding over years.
In support to Syngenta breeding, we validated the usage of supervised classification methods for automating the assignment of fingerprinted materials into genetic groups. This approach requires the preparation of a training data set containing pillar lines genotypes and their assignments in genetic groups. The stability of the training set brings stability to the results: the same model can be applied on different datasets. The classification accuracy has been estimated for different methods and several crops. We developed a tool embedding this approach and proposing specific visualizations for helping the breeders analyzing genetic structure of a set of inbred lines.

Introgressiomics: a new paradigm for crop improvement and adaptation to climate change

Jaime Prohens[a], Pietro Gramazio[a], Mariola Plazas[a], María José Díez[a], Ana Fita[a], Francisco J. Herraiz[a], Adrián Rodríguez-Burruezo[a], Salvador Soler[a], Sandra Knapp[b] and Santiago Vilanova[a]

[a] *Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Spain*
[b] *Department of Life Sciences, Natural History Museum, United Kingdom*

jprohens@btc.upv.es

The expected increasing demand of plant products in the coming decades and the environmental changes and associated stresses resulting from climate change represent a formidable challenge for plant breeders. Many crops have a narrow genetic base resulting from bottlenecks during domestication and modern breeding and this restricts the diversity available to breeders. Crop wild relatives (CWRs) represent a source of diversity of interest to breeders as they are frequently tolerant to biotic and abiotic stresses and display interesting features (e.g., quality traits). Although the use of CWRs in breeding has allowed dramatic improvements in a number of crops, their utilization has mostly been restricted to a limited number of specific traits. We propose a new approach, which we denominate "introgressiomics", which consists in the "mass scale development of multiple plant materials carrying introgressions of genomes from wild related species into the genetic background of crops that may allow developing new cultivars with dramatically improved properties, in particular adaptation to climate change". Introgressiomics is aimed at the massive generation of introgression materials for present and future (unforeseen) needs and therefore is a form of pre-emptive breeding. Introgressiomics begins with the identification of CWRs from different genepools encompassing a high genetic diversity and that represent sources of variation for traits of interest, in particular those related to climate change adaptation. This requires exploration of germplasm banks and identification of gaps in CWR collections in order to select and collect, when needed, CWRs for introgressiomics. Interspecific hybridization, in particular with CWRs from the secondary and tertiary genepools, and obtaining backcross generations to the cultivated species both potentially represent major obstacles for introgressiomics due to pre-zygotic and post-zygotic barriers. Different breeding techniques, however, can be used to increase the chances of obtaining plant materials with introgressions. The recurrent use of genomics tools, such as markers scattered over the entire genome and in target genomic areas, is essential for developing multiple collections of introgression lines (ILs) each from a different CWR donor, or the creation of mixed introgression populations in which individual genotypes may have introgressions from different CWRs. The ultimate aim of introgressiomics is to provide breeders with a dramatically enlarged genetic pool from which to obtain a new generation of cultivars adapted to the challenge of the sustainable increase in the quantity and quality of crop production in a climate change scenario. We exemplify the use of the introgressiomics approach with the work we are undertaking in broadening the genetic diversity of the cultivated eggplant (*Solanum melongena*).

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Plenary session 6:
Plant - microbe interactions

Systems biology and molecular breeding of grass-endophyte symbiota

German Spangenberg

Biosciences Research, Department of Economic Development, Jobs, Transport and Resources, Victoria, Australia
School of Applied Systems Biology, La Trobe University, Bundoora, VIC, Australia

german.spangenberg@ecodev.vic.gov.au

Genotype-specific seed microbiota of different wheat accessions and their functional characteristics

Friederike Trognitz [a], Katja Piller [a], Andreas Börner [b], Manuela Nagel [b], Angela Sessitsch [a]

[a] *Health & Environment Department / AIT Austrian Institute of Technology, Austria*
[b] *Genebank Department / Leibniz Institute of Plant Genetics and Crop Plant Research, Germany*

friederike.trognitz@ait.ac.at

In the following decades, an increase in food demand is projected because of the rapidly increasing world population and changing consumption habits. In the meantime, less arable land will be available due to human activities and climate change. To ensure stable food production, available resources have to be used efficiently and equitably and with a concurrent reduction of food waste. In many regions including Europe, food production is on the verge of exceeding environmental limits. Nitrogen synthesis exceeds the planetary boundary by a factor of four and phosphorus use has reached already the planetary boundary (http://ec.europa.eu/environment/eussd/food.htm). Sustainable agricultural practices should be developed to ward off to reduce the negative impact of chemicals, like inorganic fertilizers and pesticides. Novel approaches have been proposed including the use of plant-associated microorganisms to improve plant growth and health.

Different plant and plant genotypes actively recruit members of the soil microbial community for positive feedback (Pérez-Jaramillo et al. 2015). The mechanism behind the selection is to a great extent unclear. During domestication, the genetic diversity of modern crop plant species decreased, which may affect the ability to build up beneficial association with microbes (Hacquard et al. 2015). Also intensive agricultural practices have shown to negatively influence the diversity of bacterial communities in the soil. Until know, little is known about the perfect plant-microbe system, however, single isolates from varies sources have been shown to increase biomass production, improve plant health and fitness.

Analyzing the seed microbiome of different summer wheat accessions from the Genebank we could show that the composition of the microbiome differs with accession, however, a core microbiome could be detected. Most abundant bacterial classes were Bacilli, Alpha-, Beta-, Gammaproteobacter and Actinobacteria. Additionally, seed endophytes were isolated from the different wheat accessions and tested for germination improvement in different genotypes. Some isolates improved the germination in all tested varieties to some degree, where other isolates only improved the germination of one genotype. Moreover, some isolates showed antifungal activity against *Fusarium* spp. which makes them suitable as candidates for biological control against damping off diseases and *Fusarium* head blight. Field tests revealed that after seed inoculation with selected strains, winter wheat was protected to some degree against *Fusarium* head blight.

References:

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Plant-growth promoting and drought tolerance traits of bacteria isolated from highly drought resistant *Pistacia terebinthus*: a comparison between spring and autumn isolated communities

Iva Cholakova[a], Nele Weyens[a], Andon Vassilev[b] and Jaco Vangronsveld[a]

[a] *Centre for Environmental Sciences, Hasselt University, Belgium*
[b] *Department of Plant Physiology and Biochemistry, Agricultural University of Plovdiv, Bulgaria*

iva.cholakova@uhasselt.be

Plants exposed to drought show inhibited growth and less biomass production due to the water shortage. The importance of water for all physiological processes of the plant makes it essential. However, large parts of the world suffer from inappropriate amounts of water resources. A possible solution is the use of plant-associated bacteria (rhizosphere bacteria and endophytes) which possess different mechanisms to promote plant growth and to assist plant's survival in dry conditions. Bacteria can enhance biomass production of plants by providing nutrients in a form that can be used by plants such as ammonia (bacterial nitrogen fixation), phosphates (bacterial phosphate solubilisation) and iron (bacterial siderophores increase iron uptake). Another mechanism bacteria use to affect plant growth and development is the production of plant growth hormones of the auxin class such as indole-3-acetic acid (IAA) or by decreasing the levels of stress hormones such as ethylene (bacterial production of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase that cleaves ACC, the precursor of ethylene). Bacteria can protect plants from severe environmental conditions by producing osmoprotectants such as proline (increases survival under extreme osmotic stress) or by producing exopolysaccharides (EPS), which act as a boundary between plant's cells and the harsh surrounding environment.

Plant (leaf, stem and root from highly drought tolerant *Pistacia terebinthus* trees) and soil (rhizosphere and bulk) samples were collected in spring and autumn from an arid region in Bulgaria. The collection of isolated cultivable bacteria consists of 211 spring isolates and 273 autumn isolates. All of the isolates were tested for their plant-growth promoting capacity using 5 tests – production of IAA, ACC-deaminase, siderophores, fixation of nitrogen and solubilisation of phosphates. The strains showing positive outcomes for all 5 tests (25 for spring and 47 for autumn isolates) were further investigated for their drought tolerance using 2 tests – production of EPS and proline. All spring isolates were positive for both drought tolerance tests, but among autumn isolates only 18 were capable to produce both EPS and proline. The most promising strains based on these *in vitro* tests (25 spring and 18 autumn isolates) were genotypically characterized by sequencing of the 16S rDNA. The latter results showed that the most promising spring isolates belong to 8 genera and most represented are *Pantoea*, *Pseudomonas* and *Arthrobacter*. The most promising autumn isolates belong to 7 genera (4 in common with spring isolates) and most represented are *Pantoea* and *Raoultella*. All 43 strains were used in an inoculation experiment with *Agrostis stolonifera* seed germination, with and without drought exposure, to evaluate whether the *in vitro* beneficial effects are occurring *in planta* as well.

Breeding for symbioses – Mycorrhizae as a case study

Pierre Hohmann[a], Gunter Backes[b], Cécile Thonar[a] and Monika Messmer[a]

[a] *Department of Crop Sciences, Research Institute of Organic Agriculture (FiBL), Switzerland*
[b] *Faculty of Organic Agricultural Sciences, Kassel University, Germany*

pierre.hohmann@fibl.org

Plant associated soil microbes are known to play an important role in the expression and stability of certain plant traits such as nutrient use efficiency and disease resistance. Arbuscular mycorrhizal fungi (AMF) form one of the primary mutualistic plant-microbe symbioses. Besides known benefits such as improved nutrient mobilisation (mainly phosphorus and zinc) and tolerance against abiotic stresses (mainly drought), an increasing number of studies highlight a significant role of AMF in the mediation of disease resistances and priming mechanisms. Individual reports have shown enhanced levels of defence-related compounds (such as glucanases, chitinases and phenolics) in mycorrhizal plants, and there is first evidence of certain phytohormone pathways (in particular jasmonate signalling) to be involved in mycorrhiza-mediated disease resistance.

The level of mycorrhisation (formation of mycorrhizae on the roots) and mycorrhizal responsiveness (response to AMF) can vary widely between plant species and also among genotypes within the same species, indicating a genetic basis for the regulation of this symbiosis. Genotypic differences in mycorrhizal responsiveness have been observed in various crops and quantitative trait loci (QTL) that govern plant growth responses to AMF have been reported for maize, barley and onion. However, little is known about the heritability of mycorrhiza-mediated disease resistance. Mycorrhizal responsiveness (when based on biomass) is negatively correlated with available soil P content. Breeding under high P conditions might therefore indirectly select for poor AMF hosts. We hypothesise that a reduced mycorrhizal dependency also affects other benefits elicited by AMF such as disease resistance. We therefore pledge to include factors other than biomass to estimate mycorrhizal responsiveness (i.e. disease resistance, PUE and drought tolerance) to obtain a more comprehensive differentiation of the plant-AMF interaction. The authors also propose to complement mycorrhizal responsiveness with an additional measure called mycorrhizal efficiency since mycorrhization and mycorrhizal responsiveness on their own might not indicate an optimum cost-benefit ratio of this symbiosis. We will present initial results on genotypic variation in mycorrhization, mycorrhizal responsiveness and mycorrhizal efficiency of SNP-genotyped accessions of pea (*Pisum sativum* L.). Eventually, these SNP-genotyped accessions can be used to identify QTL that govern mycorrhiza-mediated disease resistance and exploit genotypic differences, e.g., via marker-assisted selection.

Another research project has been initiated to investigate the role of flavonoids in defence mechanisms of pea and their possible function in microbe-mediated disease resistance. Variation in microbial composition has been attributed to a differential exudation of compounds that stimulate or suppress particular members/groups of the microbial community. The complex group of flavonoids has been shown to play a signalling and/or direct role in plant defence mechanisms, but also to influence the interaction with symbionts including mycorrhizal fungi (and also plant-symbiotic rhizobia). Overall, current and future research activities of our group aim to better understand and make use of plant-microbe interactions in plant breeding for an improved expression and stability of important plant traits.

Epichloe endophyte increases seed set in tall fescue through self-pollination

Maryam Zarean, Aghafakhr Mirlohi, Mohammad R. Sabzalian

Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology , Iran

Zarean.maryam@gmail.com

Seed set through self-pollination may play an important role in maintaining plant genotypes and generating uniform plants in tall fescue (*Festuca arundinacea = Syn. Schedonorus arundinaceus and Lolium arundinaceum*) as a self-incompatible species. On the other hand, tall fescue has symbiotic association with an endophytic fungus (*Epichloe coenophiala*) which improves its forage and seed yield and protects the plant against pests and diseases. Any changes in plant genotype due to cross-pollination may decrease the effect of endophytic fungi. The present experiment was aimed to evaluate the effect of this endophytic fungus on self and cross pollination, seed set and seed characteristics of tall fescue. Plant materials included two tall fescue genotypes, 75B and 75C, collected from rangelands of Iran both containing the endophytic fungus *Epichloe coenophiala* (E+). The endophyte free plants (E-) were obtained by removing the fungus from the E+ clones using fungicides according to reported literature. Both E+ and E- plants were planted in the field in three replications. Nine panicles from each genotype were placed in paper bags before flowering and obligate self-pollination was applied. Other plant panicles were left to be open-pollinated. The number of seeds in self-pollinated panicles, number of seeds in open-pollinated panicles, self-pollination percentage, seed thickness, 100-seed weight in self-pollinated panicles, 100-seed weight in open pollinated panicles and the ratio of seed numbers in self-pollinated panicles to seed numbers in open-pollinated panicles were evaluated. The results of analysis of variance showed that genotypes*endophyte interaction was significant for the number of seeds in open-pollinated panicles and the percent of seed set due to self-pollination. Results of mean comparisons indicated that endophyte infection increased seed set through self- and cross pollination in tall fescue. Presumably, this may have resulted from changes in hormone balances induced by endophytic fungus which in turn may have improved plant seed and forage yield. Interestingly, a significant percent of self-fertilization (23-60%) was found in E+ genotypes while the percent of self-fertilization in E- counterparts was nil (0.00%).This shows that the presence of endophyte increases seed productivity through self-pollination. However, self-fertilization decreased seed thickness and 100-seed weight possibly due to inbreeding effect. It is concluded that tall fescue genotypes infected with compatible endophytic strains could be utilized for seed production via self-pollination.

Plenary session 7:
Innovation vs. regulation

Innovation vs. regulation – Maintaining biodiversity and breeding innovative cultivars

Richard GF Visser

Wageningen UR Plant Breeding, Wageningen University & Research, The Netherlands

richard.visser@wur.nl

Plant breeding is by definition a very innovative profession. Having rules and regulations in place is instrumental in providing seeds and planting materials of outstanding varieties. Not only for the way varieties are made and protected but also to control the quality of the starting material many countries have adopted international rules and regulations as for instance established within UPOV for variety protection. Also within many countries equalization of seed testing and certification rules exist and are enforced to prevent seed or planting material borne diseases from becoming a problem. This all is of importance for traditionally bred varieties and even gets more difficult with the increase of patents on plant materials as well as the unclear legal situation of many New Breeding Technologies which are finding their way to the breeding companies and potentially to novel varieties. Recapturing investments made in breeding is getting more and more problematic and a solution needs to be found how this can be safeguarded also in the future. With the conviction that innovation is the driving force of breeding it is clear that ways have to be found to capitalize for a certain time on new varieties to ensure further rounds of new developments.

The playing field has become even more controlled -and some people say restricted- since the recent implementation of the Nagoya protocol which puts quite a burden on the free transfer of genetic material. Standard Material Transfer agreements as well as access benefit sharing have to be in place before material can be transported and used in breeding programs. At the same time in many countries there is a demand from parts of the society to ensure that biodiversity is maintained, without realizing often what that is and means. There is furthermore a fear that biodiversity and even food production and supply are at risk, because only few companies have a large share of the market and seek to control and expand their position by issuing more and more patents and having similar or even a limited number of varieties in the market.

This presentation will discuss the issues raised above and provide ‘food for further thoughts’ on this important and complex issue.

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