

XXII EUCARPIA

Maize and Sorghum Conference

**Resources in
Maize and Sorghum Breeding**



Opatija, Croatia, June 19-22, 2011

Conference Book

EUROPEAN ASSOCIATION FOR RESEARCH ON PLANT BREEDING
EUROPÄISCHE GESELLSCHAFT FÜR ZÜCHTUNGSFORSCHUNG
ASSOCIATION EUROPÉENNE POUR L'AMÉLIORATION DES PLANTES

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Maize and Sorghum Section of

EUCARPIA

XXII Conference

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Maize and Sorghum Breeding“**

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XXII Eucarpia Maize and Sorghum Conference

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FINAL PROGRAM AT A GLANCE

Sunday, June 19, 2011

17:00-21:00 **Registration**
20:00-23:00 Welcome Party

Monday, June 20, 2011

8:00-11:00 **Registration**
9:00 - 9:30 **Welcome and Opening Address**
09:30-11:15 **Opening Keynotes**
11:15-11:30 Break
11:30-13:05 **Session 1**
13:05-14:30 Lunch
14:30-16:10 **Session 2**
16:10-16:25 Break
16:25-19:00 **Session 3**

Tuesday, June 21, 2011

09:00-11:20 **Session 4**
11:20-11:35 Break
11:35-13:15 **Session 4** (cont'd)
13:15-14:30 Lunch
14:30-16:30 **Poster Viewing – All Sessions**
17:00 Excursion and Conference Dinner

Wednesday, June 22, 2011

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11:25-11:40 Break
11:40-12:40 **Session 3** (cont'd)
12:40-14:00 Lunch
14:00-15:25 **Session 5**
15:25 EUCARPIA Meeting and Closing Remarks

SESSIONS

Session 1 - Genetic Resources

Session 2 - Genomic Databases and High-Resolution Mapping
Populations

Session 3 – Breeding for Specific Traits

Session 4 – New Tools and Technologies

Session 5 – Resource Allocation; Educational and Human Resources;
Miscellaneous

FINAL PROGRAM

Sunday, June 19, 2011

- 17:00 – 21:00 **Registration**
- 20:00 – 23:00 Welcome Party

Monday, June 20, 2011

- 08:00 - 09:00 **Registration and Poster Hanging**
- 09:00 - 09:30 **Welcome and Opening Address**

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- 09:30 – 10:05 **Schnable P.S.**, Liu S., Yang J., Ying K., Nettleton D., Lai J., Wang J., Springer N., Jeddloh J.
The maize genome: New tools, complexities and opportunities
- 10:05 – 10:40 **Melchinger A.E.**, Grieder C., Riedelsheimer C., Czedik-Eysenberg A., Lisek J., Sulpice R., Altmann T., Stitt M., Willmitzer L.
Breeding maize for biogas production: Genetic variability for relevant traits and their prediction by phenomic and genomic tools
- 10:40 – 11:15 **Vandenhirtz J.**
High-throughput phenotyping – A boost for genomics in the 21st century
- 11:15 – 11:30 Coffee/Tea Break

Session I: Genetic Resources

Chairs: **Berenji J., Dahlberg J.**

- 11:30 – 12:05 **Dahlberg J.**, Berenji J.
Assessing sorghum germplasm for new traits: Food and fuels
- 12:05 – 12:20 **Muraya M.M.**, Geiger H.H., Parzies H.K.
Assessment of the potential risk of crop-to-wild gene flow in regard to introduction of GM-sorghum in Africa
- 12:20 – 12:35 **Pot D.**, Trouche G., Vaksman M., Clerget B., Bastianelli D., Chantereau J., Maleyrat J., Rivallan R., Bonnal L., Rami J.F., Barrière Y.
Association mapping of biomass and cell wall related traits in sorghum
- 12:35 – 12:50 **Leiser W.L.**, Rattunde H.F., Melchinger A.E., Parzies H.K.
*Performance and stability of sorghum (*Sorghum bicolor* L. Moench) under variable phosphorous soil conditions in Mali (West Africa)*
- 12:50 – 13:05 **Butnaru G.**, Sarac I., Ciulca S., Suba D.
The evaluation of corn landraces in ex situ conditions
- 13:05 – 14:30 Lunch

Session II: Genomic Databases and High-Resolution Mapping Populations

Chairs: **Lawrence C. J., Melchinger A.E.**

- 14:30 – 15:05 **Lawrence C.J.**
Informatics for maize research: What is possible, and what is practical?
- 15:05 – 15:40 **Jiang S.**, Ramachandran S.
Sorghum functional genomics on sucrose metabolism and its molecular improvement

- 15:40 - 15:55 **van Inghelandt D.**, Stich B., Lebreton C., Melchinger A.E.
Genetic diversity, population structure, and linkage disequilibrium in the context of genome-wide association mapping in elite maize germplasm: A case study of northern corn leaf blight resistance
- 15:55 - 16:10 **Šimić D.**, Lepeduš H., Cesar V.
Early detecting of drought stress in the maize IBM population - QTL analysis
- 16:10 - 16:25 Coffee/Tea Break

Session III: Breeding for Specific Traits (1)

Chairs: **Hochholdinger F., Motto M.**

- 16:25 - 17:00 **Bohn M.O.**, Marroquin J., Hibbard B.E., Flint-Garcia S., Uphaus J.
*Native resistance of maize against the western corn rootworm (*Diabrotica virgifera virgifera* LaConte)*
- 17:00 - 17:35 **Hochholdinger F.**
*Genetic and genomic analysis of maize (*Zea mays* L.) root stock architecture*
- 17:35 - 18:10 Hartings H., Lauria M., Rossi V., **Motto M.**
Gene discovery to improve grain quality-related traits in maize
- 18:10 - 18:45 **Martin M.**, Bolduan C., Miedaner T., Dhillon B.S., Kessel B., Ouzunova M., Melchinger A.E.
*Breeding maize for resistance to ear rots caused by *Fusarium* species*
- 18:45 - 19:00 **Parzies H.K.**, Bhosale S.U., Stich B., Hash T., Rattunde F.W., Melchinger A.E., Haussmann B.I.G.
Identification of genes involved in the control of photoperiod sensitivity - A key adaptation trait in sorghum cultivation in West Africa

Tuesday, June 21, 2011

Session IV: New Tools and Technologies

Chairs: **Charcosset A., van Eeuwijk F.**

- 09:00 – 09:35 **Albertsen M. C.**
From basic reproductive biology to novel hybrid maize seed production
- 09:35 – 10:10 **Bernardo R.,** Jung H.J.G., Massman J.M., Lewis M.F., Lorenzana R.E.
Strategies for using molecular markers to simultaneously improve maize grain yield and stover quality for ethanol production
- 10:10 – 10:45 **van Eeuwijk F.,** Zhang L., Malosetti M., Boer M.
Mixed model methodology for studying interactions of QTLs with environment and genetic background in maize
- 10:45 – 11:20 **Charcosset A.,** Huan Y.F., Moreau L.
Genetic architecture of maize grain yield and related traits in a Flint by Dent cross: Comparison of intermated and conventional populations
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- Chairs: **Geiger H.H., Marton C.L.**
- 11:35 – 12:10 **Geiger H.H.**
Genetic correlations between haploids, doubled haploids, and testcrosses in maize
- 12:10 – 12:45 **Prigge V.,** Babu R., Bänziger M., Melchinger A.E.
Doubled haploids in tropical maize: Performance of inducers, QTL mapping for haploid induction rate, and variation of fertility after induction
- 12:45 – 13:00 **Elkonin L.A.,** Tsvetova M.I.
Genetic and cytological characterization of male sterility mutations induced by ascorbic acid in sorghum tissue culture

13:00 – 13:15	Varga P. , Berzy T., Anda A., Ertsey K. <i>Relations between the shelling harvesting method and seed biological value - in case of some Pioneer hybrids</i>
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10:05 – 10:40	Zinselmeier C., Sawkins M., Betran J. <i>Improving maize hybrids tolerance to water deficit</i>
10:40 – 10:55	Vilela B. , Moreno A., Lumbreras V., Pagès M. <i>Drought signal transduction in maize: Characterization of SnRK2 kinases</i>
10:55 – 11:10	Baute J. , Nelissen H., Rymen B., Jikumaru Y., Moreel K., Hollunder J., Van Lijsebettens M., Kamiya Y., Beemster G., Inzé D. <i>The maize leaf: An ideal tool to study cell division and cell expansion, the two processes driving growth</i>
11:10 – 11:25	Pinter J. , Glenn F., Pen S., Pok I., Hegyi Z., Hadi G., Marton L.C. <i>Utilising leafy genes as resources in quality silage maize breeding</i>

- 11:25 – 11:40 Coffee/Tea Break
- Chairs: **Corneanu M., Konstantinov K.**
- 11:40 – 11:55 Babić M., Anđelković V., Mladenović Drinić S., **Konstantinov K.**
Pre-breeding and maize breeding on yield and grain quality improvement
- 11:55 – 12:10 **Bukan M.**, Šarčević H., Palaveršić B., Buhiniček I., Sabljo A., Jambrović A., Lewis R.S., Kozumplik V.
Evaluation of nitrogen use efficiency in Maksimir 3 synthetic maize population
- 12:10 – 12:25 **Kadri F.**, Nadjemi B., Boudjeniba M.
*Contribution to Algerian Sahara sorghums [*Sorghum bicolor* (L.) Moench] inventory*
- 12:25 – 12:40 **Corneanu M.**, Corneanu G.C., Danci O., Sarac I., Petcov A., Buzdugan E.
Evaluation of some Romanian maize landraces for tolerance to heavy metals (Cd, Pb, Ni) by cell division cycle investigations
- 12:40 – 14:00 Lunch

Session V: Resource Allocation; Educational and Human Resources; Miscellaneous

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- 14:00 – 14:35 **Van Waes J.**
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- 14:35 – 14:50 Simeonovska E., Gadžo D., Jovović Z., **Murariu D.**, Kondić D., Mandić D., Fetahu S., Šarčević H., Elezi F., Prodanović S., Rozman L., Veverita E., Kolev K., Antonova N., Thörn E.
Collecting local landraces of maize and cereals in South Eastern Europe during 2009 and 2010

- 14:50 – 15:25 **Zdunić Z.**, Brkić I., Jambrović A., Ledenčan T., Šimić D.
Resources for public maize breeding programs in Southeast Europe
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- 1.2 Anđelković V., Kravić N., Ignjatović-Mićić D., Vančetović J.
Maize gene bank as a source of beneficial alleles
- 1.3 Bychkova V.V., Elkonin L.A., Kibalnik O.P.
Parameters affecting photosynthetic activity of F₁ grain sorghum hybrids obtained in different types of sterile cytoplasms
- 1.4 Delić N.S., Pavlov J.M., Žilić S.M.
AMMI analysis as effective tool in development of maize (Zea mays L.) breeding concepts
- 1.5 Fetahu S., Bajraktari I., Aliu S., Rusinovci I., Kaciu S., Salihu S., Sylanaj S., Shala A., Zeka D., Behluli A.
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- 1.6 Grčić N., Babić M., Anđelković V., Vančetović J., Ignjatović-Mićić D., Stevanović M., Pavlov J.
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Settling the potential of Portuguese maize germplasm for association studies
- 1.8 Mienie C.M.S., Fourie A.P., Mashingaidze K.
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- 1.9 Murariu M., Murariu D., Placinta D.D., Leonte C., Simioniuc D.P., Leahu A., Avramiuc M.
Evaluation of Romanian maize local landraces for increasing the efficiency of their use in breeding programs

- 1.10 Pavlov J.M., Delić N.S., Stevanović M.D., Crevar M.S., Žilić S.M.
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- 1.11 Rácz F., Hidvégi S., Szőke C., Hegyi G.Z., Marton C.I.
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- 1.13 Sangiorgio S., Gabotti D., Malgioglio A., Manzotti P.S., Carabelli L., Spini A, Consonni G., Gavazzi G.
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- 3.21 Mortazavian S.M.M., Choukan R., Bihamta M.R.
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- 3.24 Palaveršić B., Kozić Z., Vragolović A., Jukić K., Jukić M., Buhiniček I.
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- 4.3 Bergonti M., Pasini L., Marocco A., Amaducci S.
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- 5.9 Ochodzki P., Warzecha R., Żurek M., Góral T.
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OPENING KEYNOTES

The maize genome: New tools, complexities and opportunities

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A project to generate a near-complete sequence of the maize inbred B73 utilized a minimal tiling path of approximately 16,000 mapped BAC clones. Project methodologies and outputs will be briefly reviewed before addressing genome-enabled investigations of genome structure and function. Following its domestication ~10,000 years ago, breeders have exploited the extensive genetic diversity of maize. The roles of structural variation, including insertions, deletions and copy number variation (CNV) on the phenotypic diversity and plasticity of this important crop have not been elucidated. Whole-genome array-based comparative genomic hybridizations (aCGH) and re-sequencing experiments have revealed substantial structural variation among maize inbreds. Hundreds of sequences exhibit CNVs among genotypes, or presence/absence variation (PAV). PAVs include single-copy, expressed, full-length genes. A model that explains the origin of recurrent *de novo* CNV will be presented, as well as evidence that PAVs contribute to phenotypic diversity. Implications for plant breeding will be discussed.

Breeding maize for biogas production: Genetic variability for relevant traits and their prediction by phenomic and genomic tools

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Maize is the most important crop for biogas production in Central Europe. A deep understanding of the interrelations of the various measurable traits related to biogas production as well as tools to predict testcross performance of major target traits are still lacking. In this study, we analyzed 300 inbred lines and their testcrosses with two European flint F₁ testers during two years at three locations in Germany for line *per se* and testcross performances for agronomic and quality traits related to biogas production such as specific methane yield (SMY) and chemical composition. The lines were genotyped with a 56k SNP chip and subjected to a comprehensive metabolic profiling at early development. Biomass accumulation was monitored with a high-throughput system. Agronomic traits like dry matter yield had stronger correlation with total methane yield than quality traits such as SMY, suggesting that selection for dry matter yield is more efficient for increasing total methane yield. High genomic prediction accuracies for GCA effects were obtained for dry matter yield (0.73) and were even higher when combined with line *per se* performance (0.83). Prediction accuracies for chemical composition traits reached similar values. Metabolites showed prediction performances well above those reported previously but carried no additional predictive gain when combined with SNP markers. Our results showed that genomic prediction of GCA of dry matter yield is most promising for enhancing total methane yield. In this study, we established a comprehensive data base for the allocation of resources to phenomics, genomics and metabolomics in energy maize breeding programs.

High-throughput phenotyping – A boost for genomics in the 21st century

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Due to the development of highly automated genetic analysis, plant genomics has immensely enlarged our understanding of the genetic structure of plants over the last two decades. The fast evolving need to identify interactions between genes and environmental factors (biotic and abiotic) that brings about a certain plant phenome made it necessary to develop quantitative, reproducible and highly automated plant phenotyping systems for large plant numbers. Phenotyping systems such as these have to integrate reproducible plant management (randomization, watering) and comprehensive imaging of root and shoot far beyond human vision (visible light, fluorescence, near infrared, infrared, X-rays, THz) as well additional chemical analysis methods. Immediate and automated image analysis of the stored images and further data transformation using plant shape and plant growth models are the important intermediate steps before undertaking statistical data analysis of the phenotyping results to characterize plant phenotypes quantitatively. Such quantitative data contributes in a decisive way to the further analysis of gene functions (tilling, QTL etc.), especially under fluctuating or stress-induced environmental conditions with a special focus on complex traits like yield or drought tolerance. This presentation will provide a survey on phenotyping technology and the close interaction between phenotyping technologies, modeling approaches and the new opportunities of fast and automated high-throughput genomics.

ORAL PRESENTATIONS

Session I

Genetic Resources

Assessing sorghum germplasm for new traits: Food and fuels

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Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop in the world; however, it has a wide range of other applications that are being explored with worldwide interest in renewable resources. The U.S. sorghum germplasm collection contains over 41,000 accessions that represent landraces and cultivars from over 115 countries. Traditionally, this collection has been evaluated for such traits as abiotic or biotic stress and other agronomic characteristics and little work has been done on the collection to characterize it for what might be considered “non-traditional” uses. Sorghum is a gluten-free cereal and recent research has begun to evaluate it for flour characteristics that would make it more amenable to baking and other processing technologies. New technologies are allowing sorghum germplasm to be screened for high levels of antioxidants that show promise in cancer research and glycemic control. Most recently, various forage accessions have been evaluated for their potential for renewable fuel production. Near infrared technologies have been developed to quickly and cost-effectively screen large numbers of accessions for such compositional characteristics as ash, lignin, glucan, xylan, galactan, and arabinan, all of which have unique properties related to various bioconversion technologies. Given its genetic variability, a known genomic sequence, and a robust seed industry, new utilization of sorghum within the health food market and the bioenergy arena make sorghum an attractive renewable resource for future generations.

Assessment of the potential risk of crop-to-wild gene flow in regard to introduction of GM-sorghum in Africa

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Gene flow from cultivated crops to wild relatives is undoubtedly a risk, particularly for genetically modified (GM) crops. There are mainly two potentially harmful effects of GM-crop-to-wild gene flow in sorghum: evolution of aggressive weeds (posing a serious threat to farm productivity) and possibility of extinction of wild sorghum (leading to loss of important gene reservoirs). Biofortified GM sorghum has already been developed for commercial use in Africa and its release is imminent. The need to build essential ecological data on potential adverse effects is both urgent and justified, particularly as sorghum occurs sympatrically with its wild relatives in Africa and is interfertile. In our study we assessed (1) crop-to-wild gene flow under agricultural production systems (2) wild-crop pollen competition and (3) sorghum mating system, and genetic structure. Results showed that sorghum is extremely diverse phenotypically and genetically with geographical differentiation. Primary conditions for spontaneous hybridisation are frequently met, potential for crop-to-wild gene flow exists, and transgressive segregants in advanced generations signify potential risks for novel gene transfer. Sorghum exhibits a mixed mating system, with wild sorghum displaying high outcrossing rates. We revealed that published estimates of gene flow using male-sterile plants overestimate gene flow and that pollen competition may be a significant factor influencing outcrossing rates which should be considered assessing potential risks of GM-sorghum. A near identical pattern of spatial genetic structure in cultivated and wild sorghum was observed with medium to long distance seed-mediated gene flow. Findings have implications for conservation of genetic diversity and plant breeding.

Association mapping of biomass and cell wall related traits in sorghum

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Sorghum is among the world's most important cereals in terms of human and animal nutrition and it is currently considered as a promising energy crop. In this study, the variability of biomass yield, composition (soluble sugars, lignin, cellulose, hemicelluloses) and properties (biomass and cell wall degradability), that constitute key targets for biomass quality optimization, was characterized for 410 accessions representing sorghum worldwide diversity. These evaluations were based on 5 field-experiments and biomass characteristics were estimated based on NIRS calibrations. The broad-sense heritabilities, which ranged from 0.5 to 0.9, associated with medium levels of phenotypic variability and the absence of negative genetic correlation between biomass yield and composition indicated that significant genetic gains can be achieved in the medium term. In order to reach a better understanding of the genetic determinism of these traits, 1122 sequenced DArT markers were genotyped on 177 genotypes representing the cultivated sorghum diversity. For lignin content, known to be the main limiting factor of cell wall degradability, 8 marker-trait- associations were detected (pvalue<0.0001 in one trial and <0.05 in at least one additional trial). In parallel, 23 genes from the lignin biosynthesis pathway were sequenced on a discovery panel of 24 genotypes allowing the detection of more than 500 SNP. Haplotype SNP tags were genotyped on the whole association population (410) and association mapping analysis performed. These results are expected to provide a solid framework towards the development of innovative strategies for sorghum breeding dedicated to biomass and animal feed production.

Performance and stability of sorghum (*Sorghum bicolor* L. Moench) under variable phosphorous soil conditions in Mali (West Africa)

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A growing world population juxtaposed with diminishing phosphorus resources presents new challenges for current and future plant breeding. Soils in Sub-Saharan Africa are mostly poor of plant available phosphorus. Fertilizers are often not available or too expensive. Sorghum is the second most grown crop in West-Africa and is especially important for low-fertility soils. Plants have developed different adaptation strategies to accommodate low phosphorus conditions. A four year multi-location experiment was conducted in Mali, West Africa. Seventy sorghum genotypes (breeding-lines, landraces) were evaluated under low and high P conditions for vigor, plant height, date of heading and grain yield. The objectives were to identify well adapted and stable genotypes for low P conditions and set breeding strategies based on genotype-by-environment interaction patterns. Under low P conditions grain yield, vigor, date of heading and plant height were significantly decreased. High and low P environments showed similar broad sense heritability and genetic variation. A cross-over genotype-environment interaction was detected across low and high P environments. Grain yield in high and low P was highly correlated, but direct selection in low P was superior to indirect selection in high P. Dynamic and static stability was evaluated within high and low P environments. Index selection with AMMI stability value (ASV), response coefficient b and best linear unbiased prediction (BLUP) for grain yield seemed to be an appropriate approach for selecting stable high yielding genotypes for low input conditions.

The evaluation of corn landraces in *ex situ* conditions

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Our constant research aim was to collect, to preserve and to evaluate corn landraces in *ex situ* conditions. The 25 analyzed landraces were collected during 1975 - 1988 on Hunedoara County territory in different mountain areas. Since the collection data the landraces diversity was preserved using SIB technique. Further tasks are to develop researches to point out corn plant and yield traits stability and durable resistance to abiotic and biotic stress factors. The yield traits stability was evaluated during 6 years (2005 – 2010) in Timisoara *ex situ* conditions. The experimental climatic conditions were favorable for corn cultivation in 2005 and 2008 and improper in 2007 and 2009. The stability of cob length, kernel number/cob, cob/weight and TKW were analyzed by different methods (Finlay & Wilkinson, 1963; Wricke, 1964; Hardwick & Wood, 1972 and Gauch & Zobel, 1988). The results of each trait are discussed. The cob length was at maximum level in 2005 and 2006. The smallest cob length was in 2007 and 2009. Having in account the IPCA1 axis the lowest variability was in 2007. In the same appreciation the variability was higher in 2005 and 2006. In these 2 years the cob length was higher than the general experience average. The interaction of first principal component axis (IPCA1) pointed out a high stability in land races 697-Bacaia, 670-Mada, 671-Renghet, 493-Balsa and 698-Gradistea, correlated with a superior cob length to experimental average.

Session II

Genomic Databases and High-Resolution Mapping Populations

Informatics for maize research: What is possible, and what is practical?

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The informatics tools and technologies developed to address problems in fields outside of biology often drive what becomes available to biologists. Within the biological sciences, research groups have made headway implementing tools to solve problems of interest to maize researchers, but we do not have all the best tools deployed for maize research in an easy-to-use fashion and with personnel in place to maintain those resources. Here I describe some tools and other resources that appear interesting and useful for maize research that are available for implementation, and outline items that are really needed now that could be deployed and made available within the next few years.

Sorghum functional genomics on sucrose metabolism and its molecular improvement

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Sweet sorghum is a biofuel crop of growing importance for ethanol production. We are focusing on further improving sweet sorghum varieties in their stalk sugar content and biomass yield using traditional and molecular breeding strategies. The grain sorghum genome has been sequenced and publicly available. We have jointly launched and finished the re-sequencing program of additional three sorghum varieties including sweet sorghum (unpublished). Thus, bioinformatics approaches have been employed to identify key genes related to sucrose metabolism on a genome-wide level. Currently, we have identified total of 57 sucrose metabolism related genes from 9 different gene families. They encode 9 enzymes including fructokinase, hexokinase, sucrose invertase, phosphoglucomutase, phosphoglucose isomerase, sucrose phosphate phosphatase, sucrose phosphate synthase, sucrose synthase, and UDP-glucose pyrophosphorylase. Comparative genomics studies between grain and sweet sorghum showed that some of sucrose metabolism related genes are very conserved and only limited variation has been detected. Generally, around 60-80% of genes in these nine families can be detected with variations either in single nucleotide polymorphism (SNP), insertion/deletion (Indel) or structural variation (SV). They also exhibited the difference in exon splicing between grain and sweet sorghums. Comparative expression analysis revealed several genes with differential expression profiling between grain and sweet sorghums. In addition, these sucrose metabolism related genes also exhibited the difference in response to sucrose treatment between grain and sweet sorghum, suggesting the divergence of sucrose metabolism pathway between these two species. These genes are currently being utilized for molecular improvement by transgenic approaches through over or down expression.

Genetic diversity, population structure, and linkage disequilibrium in the context of genome-wide association mapping in elite maize germplasm: A case study of northern corn leaf blight resistance

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Setosphaeria turcica is the causal pathogen of northern corn leaf blight (NCLB) in maize that can lead to dramatic yield reduction. Association mapping is promising to unravel the genetic basis of complex traits. Information about the genetic diversity, population structure, and linkage disequilibrium (LD) in the germplasm under consideration is of fundamental importance to judge its applicability. The objectives of our study were to (i) examine population structure, genetic diversity, and LD in elite maize germplasm based on simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers, (ii) estimate the number of SNP markers needed to perform genome-wide association analyses, and (iii) identify chromosomal regions affecting flowering time and NCLB resistance in European elite maize germplasm. Our study was based on 1,537 elite maize inbred lines genotyped with 359 SSR and 8,244 SNP markers. These inbreds have been phenotyped for flowering time and NCLB resistance from 2000 to 2009 in multi-location field trials. Our results suggested that the same conclusions regarding the structure and the diversity of heterotic pools could be drawn from SSR and SNP markers. However, we propose that between 7 and 11 times more SNPs than SSRs should be used to obtain population structure and genetic diversity estimates with similar precision. The SSR markers employed in our study are not adequate for association analysis, because of insufficient marker density for the germplasm evaluated. Based on the decay of LD in the various heterotic pools, between 4,000 and 65,000 SNP markers are required to detect associations with rather large QTL with a reasonable power. Results from mixed-model association analyses revealed significant marker-phenotype associations on one and four chromosomal regions for flowering time and NCLB, respectively.

Early detecting of drought stress in the maize IBM population – QTL analysis

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The intermated B73 × Mo17 (IBM) recombinant inbred (RI) maize population is a valuable resource for the analysis of quantitative traits and is the maize breeders' community standard for genetic mapping, as it has extensive recombination, and an extensive number of molecular genetic markers. Our previous study revealed that the IBM population is also recommendable for photosynthesis research. The status and functioning of the photosynthetic apparatus can be analyzed by measuring of the chlorophyll *a* fluorescence transient induced by pulse of saturating light (JIP-test). The JIP-test is particularly useful for probing the vitality of crop plants under water-limited conditions, thereby early detecting drought stress under field conditions. The aim of the present study was to conduct a quantitative trait loci (QTL) analysis for six JIP-test parameters (ABS/RC, TR₀/RC, ET₀/RC, DI₀/RC, Fv/Fm and PI) in order to elucidate the genetic and physiological mechanism(s) in maize during flowering under water-limited and well-watered field conditions. The IBM maize population of 206 recombinant inbred lines was mapped using set of 636 molecular markers across the whole maize genome. QTL analysis revealed 40 significant QTLs for six JIP-test parameters under water-limited conditions and 57 significant QTLs under well-watered conditions. None of these 97 QTLs were detected in both environments indicating significant locus by environment interactions for all six JIP-test parameters. Many of the detected QTLs are co-localized in each environment, particularly QTLs for ABS/RC, TR₀/RC and DI₀/RC. Weak correlations with other JIP-test parameters in both environments, as well as completely different set of detected QTLs compared to other parameters highlighted ET₀/RC (electron transport per reaction center) as a genetically distinctive photosynthetic parameter. QTL analysis was capable to identify several candidate genes possibly associated with chlorophyll *a* fluorescence parameters. Our results suggest that the improvement of drought tolerance by marker-assisted selection for JIP-test parameters in maize is feasible.

Session III
Breeding for Specific Traits

Native resistance of maize against the western corn rootworm (*Diabrotica virgifera virgifera* LaConte)

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During the last 60 years more than 12,000 maize accessions, comprising inbreds, synthetics, and open pollinated varieties, as well as maize relatives, like teosinte and tripsacum, have been screened for their level of resistance to western corn rootworm (WCR) larvae feeding. Less than 1% of this germplasm was selected for initiating recurrent selection programs. In general, the selected genotypes were characterized by large root systems and superior secondary root development after root damage caused by WCR larvae. However, no non-transgenic maize cultivars with high level of resistance under moderate to high insect pressure have yet been released. To overcome this problem, we are in the process of evaluating the defense response of maize to WCR feeding in a coherent framework, which includes gene expression and metabolite analyses. In addition, we investigate the genetic basis of WCR resistance in new maize materials with improved levels of resistance using linkage disequilibrium mapping approaches. Two populations of testcrossed double haploids (DHs) derived from crosses between resistant and susceptible maize inbreds were evaluated for their level of resistance in three to four different environments. For each DH testcross an average root damage score was estimated and used for QTL analysis. QTL positions were identified using composite interval mapping. Significant QTL were located on chromosomes 1, 2, 7, 8, 9, and 10. A model fitting all QTL simultaneously explained about 30% of the phenotypic variance for root damage scores in both mapping populations. How this knowledge impacts the design and efficiency of breeding programs to improve WCR resistance will be discussed. As a direct output of this project, molecular markers will be available to efficiently screen germplasm for novel defense response variants and to perform marker-assisted selection.

Genetic and genomic analysis of maize (*Zea mays* L.) root stock architecture

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The maize (*Zea mays* L.) root system is shaped by various root-types that are continuously formed during development. While primary and seminal roots which are laid down in the embryo prevail shortly after germination, the adult root stock is determined by shoot-borne roots that are formed at consecutive stem nodes. Recently, a collection of maize mutants with specific defects in different root-types has been assembled and some of the affected genes have been cloned. Among these two genes are involved in auxin signal transduction. *RTCS* regulates shoot-borne root initiation and encodes an auxin inducible LOB domain transcription factor, while *RUM1* which is required for lateral and seminal root initiation, encodes a member of the AUX/IAA gene family. The ongoing functional characterization and molecular interactions of these genes and the encoded proteins will be discussed.

Gene discovery to improve grain quality-related traits in maize

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Developing plants with improved grain quality-traits involves overcoming a variety of technical challenges inherent in metabolic engineering programs. Advances in plant genetics and for large-scale gene expression analysis are contributing to the acceleration of gene discovery for products development. To increase our understanding of the key molecular determinants controlling carbon flux to the grain and the partitioning of carbon to starch and proteins, we assayed a series of endosperm mutants by evaluating protein, amino acid composition, and transcriptome profiling. Specifically, for the *o2* and *o7* mutations we found that the overall amino acid compositions of these mutants appeared similar. Each mutant had a high Lys and reduced Glx and Leu content with respect to wild-type. Gene expression profiling allowed us to identify a series of mutant-related up-regulated (17.1%) and down-regulated (3.2%) transcripts. Several differentially expressed ESTs homologous to gene encoding enzymes involved in amino acid synthesis, carbon metabolism, in storage protein and starch metabolism, in gene transcription and translation processes, in signal transduction, and in protein, fatty acid, and lipid synthesis were identified. Our analyses demonstrated that *o2* and *o7* mutants are pleiotropic and play a critical role in several endosperm metabolic pathways. Although, work is required to define gene functions and dissect the complex regulation of gene expression, the genes isolated and characterized to date give us an intriguing insight into the mechanisms underlying endosperm metabolism.

Breeding maize for resistance to ear rots caused by *Fusarium* species

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Toxigenic *Fusarium* species cause different diseases in maize (*Zea mays* L.). In Europe, Gibberella ear rot (GER) is mainly caused by *F. graminearum* Schwabe and Fusarium ear rot (FER) mainly by *F. verticillioides* (Sacc.) Nirenberg. Both ear rots lead to contamination of the grains with different mycotoxins, which adversely affect the health of humans and animals. Breeding maize for resistance to these ear rots and to the associated mycotoxins is the most promising option for a sustainable solution to this problem. Here we give an overview about recent findings in resistance breeding to GER and FER and point out possibilities to implement them in hybrid breeding programs. Classical phenotypic selection for resistance to GER and FER is expensive because of the need to conduct multi-environment testing and laborious artificial inoculations. An increase of selection efficiency might be achieved by a combination of classical phenotypic and marker-assisted selection based on QTL for GER resistance and reduced mycotoxin contamination, which were identified recently in multiple crosses of flint lines. Furthermore, costs should decrease by indirect selection for reduced mycotoxin contamination based on ear rot ratings, a consequence of colocalized resistance QTL resulting in strong genotypic correlations between these traits. As correlations between line and testcross performances tend to be moderate or low, field testing should focus on testcrosses. Only few elite sources of superior disease resistance are available, but sustainable improvement of resistance, e.g. by recurrent selection, should be possible without detrimental effects on other important traits, such as grain yield.

Identification of genes involved in the control of photoperiod sensitivity - a key adaptation trait in sorghum cultivation in West Africa

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Sorghum [*Sorghum bicolor* (L.) Moench] is major cereal staple crop in West and Central Africa (WCA) where it is mainly cultivated in the Sudanian zone. Photoperiod sensitive flowering is a key adaptation trait which enables farmers to achieve more yield stability under unpredictable climatic conditions as present in WCA, particularly at the beginning of the growing season. To investigate the effect of single nucleotide polymorphisms (SNPs) on flowering time variation 219 sorghum inbred lines derived from WCA landraces were evaluated for flowering time under field conditions at two planting dates to enable calculation of a Photoperiod Response Index (PRI). To investigate marker/trait associations between SNP-variation of candidate genes involved in the photoperiodic pathway of flowering time and PRI of all sorghum lines, an association study was carried out. To avoid spurious associations, firstly population structure was assessed employing a genome-wide sample of molecular simple sequence repeat markers. The results showed that the accessions were structured into two subgroups. Subsequently, all accessions of sorghum were sequenced for SNPs in 12 candidate genes. Considering population structure and applying a mixed model approach, significant associations between several SNPs and the PRI were found for two candidate genes. Negative values of Tajima's D were observed suggesting that purifying selection has acted on candidate genes involved in photoperiodic control of flowering time in sorghum. Results are valuable for the annotation of candidate genes and to design simple diagnostic markers for photoperiod sensitivity to be used in marker-assisted breeding which is expected to accelerate breeding accomplishments.

Genetic architecture of grain carotenoids and inflorescence architecture of maize – a tale of two traits

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We study various maize traits, some as model systems and some as agronomic applied systems. We initially chose to study carotenoids because the biochemical pathway was very well characterized, particularly in lower organisms, which we felt would lead to cloning genes in the carotenoid pathway of maize. We decided to study inflorescence architecture because tassel branch number was simple to measure and the scores are integers. This is in contrast to grain carotenoids for which we obtain concentrations with continuous variation and error variances resulting from the extraction and high performance liquid chromatography processes. A candidate gene approach guided by the carotenoid biochemical pathway was very successful in identifying *lycopene epsilon cyclase (lcyE)* underlying a major QTL influencing flux down the alpha- and beta- branches of the pathway and the *beta-carotene hydroxylase crtRB1* underlying a major QTL influencing levels of beta-carotene. In contrast, use of candidate genes from cloning classic inflorescence mutants did not reveal highly significant associations with quantitative variation in tassel architecture traits. The functional polymorphisms of *lcyE* and *crtRB1* appear to be very close to the coding regions and associated with transcript levels. For inflorescence architecture, it is possible that the functional polymorphisms of candidate genes are many kb away from the gene. Therefore inflorescence architecture traits were measured on the Nested Association Mapping population and joint linkage mapping and a genome wide association study performed. This approach does reveal associations near some of the genes defined by the classic inflorescence mutant, and associations in regions without logical candidates. A comparison of the current knowledge of the genetic architectures of the two traits will be presented. Additionally, allele specific MAS for higher provitaminsA for Africa, and QTL as donors for altering branch number in elite backgrounds to enhance pollen production, will be presented.

Improving maize hybrids tolerance to water deficit

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With water becoming an increasing limiting resource in agriculture, the existing trend of higher global temperatures and potential greater regional and seasonal climatic changes, the development of corn hybrids with better drought tolerance and water use efficiency becomes an important goal in breeding programs. A multidisciplinary approach involving breeding, physiology and molecular genetics can synergistically bring more understanding to the response of corn plants to water deficits and enable the development of more tolerant hybrids. Using molecular tools targeting metabolic pathways that are related with response to stress conditions, the search for key genes involved in these pathways is underway through functional genomics, reverse and forward genetics. With better understanding of the physiological mechanisms associated with the response of corn plant to drought and their genetic dissection it is possible to identify, transfer and select key genes and alleles within these genes that provide better stress tolerance. Syngenta Seeds has developed and launched Agrisure Artesian™ technology, the new brand name for its range of water optimized hybrids. Agrisure Artesian technology enables corn plants to use available moisture more efficiently, resulting in higher yields on drought-stressed acres including dryland and limited-irrigation farms in the western US Corn Belt and more stable yields in years of inconsistent rainfall or fields with variable soil types and moisture-holding capacity in central, southern, and eastern U.S. Corn Belt. In years of ideal rainfall, hybrids with Agrisure Artesian technology have demonstrated no yield penalty compared with hybrids without the technology. Syngenta created Agrisure Artesian technology through years of advanced molecular breeding to begin identifying and mining the many genes from the corn genome responsible for managing water use in corn. Syngenta's water-optimized hybrids combine multiple genes from this pool, all of which were identified as helping corn plants produce higher yields when available water is limited.

Drought signal transduction in maize : Characterization of SnRK2 kinases

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Abscisic acid (ABA) plays a major role in regulating several developmental and physiological processes, such as seed maturation and germination, and mediating the responses of vegetative tissues to osmotic stress. ABA levels increase in late embryo development shortly before the onset of desiccation and in vegetative tissues under water-deficit stress conditions. ABA triggers both stomata closure and water-deficit tolerance responses by regulating changes in the activity of ion channels and changes in gene expression. Protein kinases are key components for mediating cellular responses, including responses to osmotic changes. Many kinases have been described to be involved in ABA and/or osmotic signalling affecting stomata function and/or gene expression. We have isolated a maize SnRK2 kinase that is constitutive, rapidly activated by ABA and osmotic stress in vegetative tissues and that is capable of complementing the *Arabidopsis ost1-2* mutant, which is defective in the regulation of transpiration upon water stress. SnRK2 phosphorylates maize DBF1 transcription factor as well as other TFs involved in Drought responses. TFs are interesting study candidates to unravel the molecular mechanisms of SnRK2 kinases since they control cellular adaptation by regulating gene expression and represent important tools for biotechnological crop improvement.

The maize leaf: An ideal tool to study cell division and cell expansion, the two processes driving growth

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The cells within a maize leaf can be classified according to their characteristics along the leaf length axis: active cell divisions occur at the base of the leaf, and as the distance from the base increases cells will cease division and start expanding until they reach their mature cell size. This spatial gradient of growth processes allows specific enrichment of dividing, expanding or mature cells in samples taken across the growing leaf. The growth of the leaf can be monitored by the leaf elongation rate and the contributions of cell division or cell expansion can be quantified by a kinematic analysis. In this way we assessed the effects of several adverse conditions, such as mild drought and cold nights, and genetic perturbations on leaf development and more specifically on the processes of cell division and cell expansion. We integrated this cellular analysis with multiple systems-wide approaches such as transcriptome, metabolome and more recently hormone profiling in an attempt to link the cellular responses to the underlying molecular mechanisms. The latest data will be presented together with the progress of the development of tools for maize transgenic and systems biology research.

Utilising leafy genes as resources in quality silage maize breeding

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The primary goal of silage maize production is to obtain the greatest possible amount of digestible nutrients. Although it is not of the highest value in terms of starch, the leaf is one of the most important parts of silage, providing ruminants with sufficient amount of green mass and facilitating nutrient uptake. The nutrient and fibre content of leaves at harvest determine the quality of silage. In cooperation with Glenn Seed Ltd, the Agricultural Research Institute of HAS had an opportunity to use an inbred line carrying the dominant allele Lfy1. The number and surface area of leaves were measured in various hybrid combinations, and the hybrids and parents were compared morphologically. The combinations causing the highest heterosis were identified. Compared to lines without Lfy1 gene and hybrids in the same maturity group, these lines had an additional 3-4 leaves above the ear on average, resulting in an extra 30-40% leaf area. The hybrid Siloking /Lfy/ produced 50% more leaves above the ear than the standard, leading to an extra 40% efficient leaf area. The results demonstrate that the increased total leaf area of Lfy silage hybrids and the number of leaves above the ear, being a crucial factor for silage production, ensure that new leaves with high sugar content capable of photosynthesizing can develop until the end of grain filling. The substantial biomass produced by Lfy hybrids may be utilized, bearing in mind the relevant guidelines by the European Union, also as biogas raw material in sustainable energy management.

Pre-breeding and maize breeding on yield and grain quality improvement

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Broad genetic variability of starting biological material is crucial prerequisite for the successful breeding program. Maize Research Institute, owning Gene bank with more than 6.000 accessions consisted of the local and introduced collection, has the opportunity for the investigation both fundamental genetic processes, pre-breeding and breeding for commercial purposes. To search for new sources of maize drought tolerance Gene bank accessions were scored visually on stay-green phenotype and total appearance. More than 50 genotypes have been identified as a potential source for drought tolerance. Besides breeding on improved storage protein quality decreasing of phytate content in kernel is also under the consideration, because the most phosphorous is bound in phytate. In breeding program it is desirable to have genotypes with higher content of available phosphorous (P) not bound in phytate. Among 60 analyzed populations from Gene bank only one has been determined to have very low phytate content and will be used in maize breeding program on low phytate in kernel. Process of maize breeding, to get high yielding hybrids, begins by the genetic variability determination of starting breeding material, either populations or selected inbred lines. Genetic divergence of parental inbred lines is main step to get high heterotic effect in yield after crossing. Use of different molecular markers allowed cluster analysis by use of UPGMA methods and select high number of genotypes to be included in crossing process. Pre-breeding and breeding program at Maize Research Institute "Zemun Polje" will be discussed in details.

Evaluation of nitrogen use efficiency in Maksimir 3 synthetic maize population

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Nitrogen use efficiency (NUE) has become important trait for sustainable agriculture. Traits adaptive to nitrogen limited environments present in maize landraces could not be easily implemented directly into modern maize breeding programs. Thus, the landraces could be improved by recurrent selection and intercrossed to form synthetics from which new lines could be derived and used as source of important traits for elite breeding material. Maksimir 3 Synthetic (M3S) maize population was created from landraces traditionally grown at low levels of soil fertility. The synthetic was subjected to recurrent selection for yield and disease resistance during three cycles and in addition for nitrogen use efficiency, in the fourth cycle. In order to evaluate the performance of populations from the four cycles of recurrent selection per se, their testcross and S1 progenies under high and low nitrogen input conditions, a field trial was conducted at four locations in 2010. The populations per se and their testcrosses had on the average higher value of grain yield 21%, kernel row number 3%, plant height 9%, ear height 15%, kernel depth 5%, ear length 6%, ear diameter 4%, and ear leaf chlorophyll content 30% at the high than at the low nitrogen fertilization rate. The S1 progenies responded similarly to nitrogen fertilization. The genotype x nitrogen interaction for yield was not significant indicating no specific adaptation to the nitrogen deficient environments. After four cycles of recurrent selection, significant increase of grain yield was found at both levels of nitrogen fertilization.

Contribution to Algerian Sahara sorghums [*Sorghum bicolor* (L.) Moench] inventory

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Local landraces are maintained in a dynamic process and usually tend to be well adapted to the specific environment in which they evolved. Resistance Sorghum [*Sorghum bicolor* (L.) Moench] to harsh environments such droughts and heat and biotic stresses makes it an important food resources in dry land ecosystems. The main purpose of this study was to accomplished sorghums inventory in an Sahara ecosystem. Two main sites are chosen from Tamanrasset wilaya bordering Niger and Mali contries. The sites are Ain Salah and Hoggar with 700 kilometres distance. Seventy three cultivars were collected from the sites during four years . A laboratory core collection was founded according to ICRISAT descriptor for sorghum. Nine quantitative traits (flowering dates, plant height, peduncle exertion, panicle width and length, grain size and seed weight) were measured. The cultivars showed agromorphological variation related to panicle and glumes shape, 1000 seeds weight. The durra race is predominantly present in both sites while the cultivars races were intermediate with caudatum or kafir. The guinea race which has trait to be growing in high rainfall amounts regions of Africa, was also recorded with 4% of cultivars. The 1000 grain weight showed a great variation from 14.5 g and 59.15 g. this variation was observed even within accessions of close morphological features. This inventory discern a wide diversity and allowed understanding landrace diversity which aids in deciphering evolutionary forces under domestication, and has applications in the conservation of genetic resources.

Evaluation of some Romanian maize landraces for tolerance to heavy metals (Cd, Pb, Ni) by cell division cycle investigations

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Plants landraces evolved over time under specific environmental conditions. Maize is one of the plants known as a hiperaccumulator of many heavy metals from contaminated soils. The key goal of these researches is the evaluation of heavy metals tolerance of five maize landraces collected from a polluted area, in order to select tolerant/resistant germplasm. The considered area is in vicinity of surface coal exploitation and thermo electric power plants, therefore the soil is contaminated with heavy metals and radionuclides. The collected landraces were evaluated for tolerance to heavy metals comparing with the inbred line Lv 113. Normal development of plantlets is dependent on plants meristems thus studies on cells proliferations in the meristems are of special interest. The germinated seeds, with 10 mm length roots, were exposed to heavy metals in 10 variants: Control (tap water), Cd (1.0, 4.0, 7.0 mg/l CdSO₄), Pb (50.0, 100.0, 200.0 mg/l PbNO₃), Ni (20.0, 100.0, 200.0 mg/l NiSO₄). The experiment was run in 3 repetitions. Roots for cytological investigations were harvested after 24 hours of treatment with heavy metals solutions (a complete mitotic cycle). After 7 days of treatment, cell cycle was studied by flow - cytometry. Mitotic index, aberrant division phases and micronuclei frequency, were evaluated in root meristems. The mitotic index was modified by the highest concentration of heavy metals. There were registered 2.5 – 5.5% cells with chromosomal aberrations, genomic mutations and metabolic modifications of the chromosomes, due to the affinity of heavy metal for DNA macromolecule and mitotic spindle microtubules.

Session IV
New Tools and Technologies

From basic reproductive biology to novel hybrid maize seed production

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Reproductive biology research has a long history at Pioneer Hi-Bred, not only with the first cloning of a male fertility gene in maize, but also with the identification of many genetic components that are required for controlling the expression of male fertility genes. One of the outcomes from this research has been the development of a novel genetic process to increase a nuclear genetic male sterile inbred that can be utilized to produce maize hybrids, designated as SPT (Seed Production Technology). This process utilizes a naturally occurring recessive mutation in a sporophytic gene required for male fertility to create female parent lines that are male sterile when the mutant allele is made homozygous. Full male fertility is restored in hybrid plants upon pollination of the male-sterile female parent plants with pollen from any male parent carrying a wild-type allele of the mutant male-sterility gene. The SPT process offers a reliable, cost-effective method to propagate pure populations of homozygous recessive male-steriles during female parent seed increase that are also non-transgenic for the SPT process. This unique process utilizes a unique, genetically modified maintainer line which does not produce transgenic male-sterile progeny to meet the challenge of propagating pure populations of male-sterile female parent lines that cannot be produced by standard self-fertilization techniques. The genetics and theory underlying the SPT process, and its role in hybrid seed production, will be discussed.

Strategies for using molecular markers to simultaneously improve maize grain yield and stover quality for ethanol production

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Maize stover is the most abundant source of lignocellulosic crop biomass that can be converted to ethanol biofuel in the U.S. Our objective was to optimize the use of SNP markers to simultaneously breed for high grain yield (for non-energy and energy uses) and high stover quality for ethanol production. We measured three stover-quality traits in testcrosses of the intermated B73 x Mo17 population: concentration of cell wall glucose in dry stover (Glucose); cell wall glucose released from the stover by thermochemical pretreatment and enzymatic saccharification (Glucose Release); and concentration of lignin on a cell-wall basis (Lignin). Genetic variances were significant for grain yield, moisture, stalk and root lodging, plant height, and all three stover-quality traits. Heritabilities were 0.57 for Glucose, 0.63 for Glucose Release, and 0.68 for Lignin. Genetic and phenotypic correlations among traits were generally favorable but also reflected the complexity of stover cell wall composition. We found 152 QTL, mostly with small effects, for Glucose Release and cell wall components. Responses to three cycles of selection for Glucose, Glucose Release, and Lignin were higher with genomewide selection (which utilized all markers rather than only those with significant effects) than with selection based only on significant markers. To our knowledge, this work represents the first report of an empirical breeding experiment on genomewide selection in plants. Given the absence of major QTL and the complexity of the traits, we recommend genomewide selection for the improvement of stover-quality traits for cellulosic ethanol in maize.

Mixed model methodology for studying interactions of QTLs with environment and genetic background in maize

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Mixed model methodology is a powerful tool for identifying QTLs in a wide array of contexts. As a first example, for data from multiple trials, the dependence of the QTL effects on the environmental conditions can be studied, while allowing for the dependence of polygenic and error variances and covariances on the environment (Boer et al. 2007). Second, for data from multiple offspring populations that are connected via common parents, the dependence of QTL effects on genetic background can be studied, while allowing for the dependence of polygenic and error variances on the particular offspring population (Huang et al. 2011). In this paper, we will introduce a mixed model framework for QTL analysis of multiple populations across multiple environments, where we will be able to study QTL by environment interactions and QTL by background interactions simultaneously. We illustrate our approach by data coming from the maize nested association mapping population of Buckler et al. (Yu et al. 2008).

Genetic architecture of maize grain yield and related traits in a maize Flint by Dent cross: Comparison of intermated and conventional populations

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Development of mapping populations after cycles of random intermating is an appealing option to improve the precision of QTL mapping without increasing population size. To complement the US IBM population, we developed a new intermated population, LHRF, between two early inbred lines issued from the European Flint and American Dent genetic pools, respectively. In order to evaluate experimentally the potential of this resource, comparative QTL detection was carried out in (i) an advanced intermated F3 population of 322 lines and (ii) a conventional F3 population of 300 lines, both derived from the same parental maize inbred lines and jointly evaluated in testcross progeny for dry grain yield and related traits. Genetic variance for dry grain yield was significantly lower in the intermated population compared to the F3 population. QTL confidence intervals were on average 2.31 shorter in the intermated population, illustrating the interest of intermated populations for increasing the resolution of QTL detection. However, fewer QTLs were detected in the intermated population and less than 50% of the detected QTLs were common between the two populations. Cross-validation also showed that selection bias was more important in the intermated population. This finding supports the hypothesis that QTL detected in conventional populations correspond mainly to clusters of linked QTL. The actual number of QTL involved in the genetic architecture of complex traits may be substantially larger, with effect sizes substantially smaller than in conventional populations.

Genetic correlations between haploids, doubled haploids, and testcrosses in maize

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Doubled haploid (DH) lines produced via *in vivo* haploid (H) induction have become an indispensable tool in maize breeding and research. To determine the predictive value of the performance of H and DH lines *per se* in hybrid breeding, genetically balanced sets of H and DH lines along with their testcrosses (TCs) were evaluated in field trials across four locations over two years in Germany. Suitable sets of material were provided by three collaborating breeders. Each set comprised 56 to 60 DH lines developed from an elite single cross. These DH lines were crossed with one or two flint testers and subjected to haploid induction for production of corresponding H versions. H, DH, and TC entries were grown in separate but adjacent blocks in the same field. H lines were surrounded by a mixture of inbred lines functioning as pollen source. Most H plants were male sterile but showed low degrees of female fertility. Highly significant genetic variation existed in all sets of material for all traits. Genetic correlations between H and DH lines were moderately strong ($0.5 < r_g < 0.7$) for biomass yield (BMY), stover yield (STY) and grain yield (GRY), and strong ($0.7 < r_g < 0.9$) for flowering date (FLD), vigor score (VIG), and plant height (PLH). Associations also existed between H lines and TCs, but they mostly were only moderately strong. Correlations between DH lines and TCs varied from non-significant to moderately strong for GRY, but were strong for FLD, grain dry matter content (GDM), and PLH. BMY, PLH, and VIG of H lines were weakly ($0.3 < r_g < 0.5$), but significantly, associated with GRY of TCs, whereas the corresponding correlations between DH lines and TCs mostly were moderately strong. In conclusion, at the haploid level, selection for VIG, BMY, and PLH will result in positive correlated genetic gain for various traits not only at the DH but also at the TC level. Likewise, selection at the DH level may lead to substantial progress in TC performance.

Doubled haploids in tropical maize: Performance of inducers, QTL mapping for haploid induction rate, and variation of fertility after induction

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Doubled haploid (DH) lines produced by in vivo haploid induction and subsequent artificial chromosome doubling are a simple tool to increase efficiency of maize breeding programs but adoption of DH technology is lagging behind in the tropics. Three temperate haploid inducers were evaluated in several environments in Mexico to study their suitability for tropical breeding programs. Haploid induction rates (HIR) obtained under tropical conditions were similar to those obtained with the same inducers under temperate conditions. Hence, temperate inducers can be employed for initiating DH breeding programs in the tropics, but agronomic problems may complicate their handling. New inducers better adapted to tropical conditions are being developed from crosses between temperate haploid inducers and tropical CIMMYT maize lines (CML). Preliminary results of extensive field testing in Mexico indicate that new inducers show excellent pollen and seed producing capacities, improved plant vigour, reduced susceptibility to tropical leaf diseases, and have sufficient HIR to be employed for large-scale in vivo induction of haploidy in tropical maize breeding programs. To support phenotypic selection of new haploid inducers, relevant loci controlling HIR were detected through linkage mapping in F₃ populations derived from CML×inducer crosses. Screening haploids from 90 germplasm revealed large genotypic variation for fertility-related traits. Tropical germplasm exhibiting improved haploid fertility after in vivo induction was identified which may enable avoiding the use of toxic chemicals for chromosome doubling during DH production. Practical implications and experiences from establishing DH technology for tropical maize breeding programs are discussed.

Genetic and cytological characterization of male sterility mutations induced by ascorbic acid in sorghum tissue culture

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Male sterility mutations denoted *ms-asc1*, *ms-asc2*, *ms-asc3* were found in plants regenerated from sorghum callus cultures (cv. Milo-10) subjected to oxidative stress by treatment with high concentration of ascorbic acid. These mutants were characterized by complete (*ms-asc1*) or partial male sterility (*ms-asc2*, *ms-asc3*). As far as primary target for oxidative stress is plant mitochondria the male-sterile phenotype of these mutants was expected to be the consequence of cytoplasmic mutations. However, the F₁ hybrids obtained by crossing these mutants with original line were completely fertile; in the F₂, semi-sterile plants were segregated-out. The *ms-asc2* mutation was transferred through the pollen by crossing emasculated plant of original line with restored F₁ hybrids that pointed on nuclear location of this mutation. Cytological analysis of microsporogenesis in semi-sterile plants with *ms-asc1* revealed irregularities of chromosome segregation and cytokinesis. In diads, division spindle formed at different angles to intercellular wall that resulted in formation of anomalous configuration of tetrad cells (T-shaped, linear). In majority of flowers, pollen grains degenerated at microgametogenesis. These deviations were accompanied by disturbances in tapetal cells: formation of two nuclei differing in size or micronuclei. In different flowers from the same panicle, the frequency of abnormalities varied considerably (1.0-80.0%) resulting in semi-sterile phenotype. Being self-pollinated semi-sterile plants produced in their progeny fertile, semi-sterile and sterile plants in variable ratios. In *ms-asc3* families, alongside with male sterility, dwarfness and awnless mutations were found in the late generations. The reasons of genetic instability both within the plants and in their progeny are discussed.

Relations between the shelling harvesting method and seed biological value - in case of some Pioneer hybrids

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An analysis was made of the germination ability and vigour of the seed of eight Pioneer maize hybrids, immediately after harvesting and a year later. Two experimental plots were marked out on seed multiplication fields, one of which was harvested shelled and the other as whole ears. The aim was to determine whether the seed showed any loss of germination ability when shelled mechanically. The seed was cleaned but not dressed, and then germinated according to the ISTA standard procedure in four seed analysis laboratories. In addition, the seed of two randomly chosen hybrids was divided into four fractions based on size and shape, each of which was examined separately. All the germination results were acceptable (over 90%), but a better percentage was achieved after manual shelling than after mechanical shelling. The number of abnormal seedlings, however, was lower for mechanically shelled seeds than for those harvested as whole ears. For two hybrids, harvesting whole ears had a clearly positive effect on seed quality (PR35Y65, LSD_{0.1%}; PR39R20, LSD_{5%}). In the case of the separate seed fractions, the lowest germination percentage was obtained for the medium-sized round fraction and the highest for the large flat fraction. For three hybrids the seed vigour proved to be better when harvested shelled than on the ear. The seed samples were stored in the Pioneer Hi-Bred ZRt Seed Plant (10–25°C, 50–60% RH), and the germination ability of four hybrids was analysed again a year after harvesting. For all the hybrids the germination percentage was lower for both groups than in the post-harvest analysis. Better results were obtained for all the hybrids after conventional (whole ear) harvesting than in the shelled group, but the difference was not significant. In the vigour tests the mechanically shelled group was superior for three hybrids, but again the difference was not significant. The experiments should be repeated over several years to determine whether mechanically shelling maize seed at harvest is feasible.

Session V

Resource Allocation; Educational and Human Resources; Miscellaneous

Adaptation of evaluation criteria to changing agricultural practice in maize and their impact on variety registration

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The past 50 years in agriculture a lot of progress was realised for yield, harvest security, disease resistance and quality. This was due to a combination of factors such as high input of fertilizers, availability of pesticides, better varieties in combination with an adapted crop husbandry. However nowadays under intensive agriculture in several European countries, for example silage and corn maize, a lot of negative aspects occur (N- and P- losses to ground water, loss of biodiversity, smaller rotations, higher disease pressure, lower soil fertility and soil structure). For agriculture in the future these negatives aspects have to be minimised but a high productivity has to be maintained for the cost-effectiveness. An important question hereby is: Are the actual cultivated crops such as maize (with in most cases a restricted variety assortment) adapted for this new evolution or do we need other crops- other types of varieties? In the European Community, a new variety of an agricultural crop must submit official trials for DUS (Distinctness, Uniformity, Stability) and VCU (Value for Cultivation and Use) before commercialisation. The guidelines for those tests are summarized in the European directive 70/457/EU (1970), revised in 2002 (2002/53/EU). To offer the best varieties to the market the aim of the VCU-research should be to predict the agronomical and technological value of a new variety on a reliable way in comparison with standard varieties. Variety research, especially the VCU test, lies between agricultural practice and breeding. The evaluation criteria are based on the most important characteristics for agricultural practice; they can push the breeding companies in a certain direction. On the basis of criteria for release of new varieties, variety research can contribute to a more intensive ecological agriculture, and anticipate on new situations. New criteria for maize varieties can be: better nutrient efficiency (lower input of fertilisers in practice), early vigour and cold tolerance in spring, drought tolerance, quality characteristics and disease resistance. Before new criteria are incorporated, their impact on variety release has to be studied. For the criteria for evaluation it is important that there is a good equilibrium between parameters for yield, harvest security (resistance to lodging and stalk rot), disease resistance and quality. The choice of the standard varieties is also very determining for the level of admission. In this presentation the different aspects for future variety testing for maize (silage and corn) and which parameters have to be judged in the framework of an intensive ecological agriculture will be discussed.

Collecting local landraces of maize and cereals in South Eastern Europe during 2009 and 2010

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Within the framework of SEEDNet program (South East European Development Network on Plant Genetic Resources), collecting missions in twelve countries were conducted under the regional project *Collecting local landraces of maize and cereals (wheat, barley, rye, oat, millet and buckwheat) in South Eastern Europe (2009-2010)*. The main goal of this project was preservation of local landraces of maize and cereals in the project countries with the aim to provide them available for breeding, research and other purposes at national, regional and international level. The project participants were the national teams from Macedonia (as project coordinator), Federation Bosnia and Herzegovina, Montenegro, Romania, Republic of Srpska, Kosovo, Croatia, Albania, Serbia, Slovenia, Moldova and Bulgaria. Each national team carried out the collecting missions in its own country. During 2009 and 2010, over 589 localities were inventoried in the predicted regions. The result of the collecting was the total number of 867 maize and cereals accessions collected. Maize prevailed with the total number of 637 collected accessions (in percent 73,47%), followed by 47 wheat acc. (5,42%), then barley 43 (5,07%), rye 40 (4,61%), oat 52 (5,99%), millet 8 (0,92%), buckwheat 9 (1,02%) and other species (*Sorghum sp.*, *Triticum monococcum*, *Triticum spelta*, *Triticum turanicum*, einkorn) 31 accessions (3,58%). Regarding the status of the samples, accessions are mainly landraces, local populations and old cultivars which are still grown by some farmers and used as a source of high quality animal and human food.

Resources for public maize breeding programs in Southeast Europe

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Maize breeding programs in Southeast Europe - European Corn-Belt (Bulgaria, Croatia, Hungary, Romania, and Serbia) were for decades of the second half of 20th century only in the public domain. Although not stimulating for commercial development of new technologies and traits, public research institutes of the region were making continuous enhancement of maize genetic resources all these years. The main sources for maize breeding in the region were original local Pannonian populations and varieties as well as elite adaptable US Corn- Belt material. However, limited time and funds were spent in research of high quality local germplasm per se. Some unique advantages of utilizing genetic, production, human and educational resources in public breeding institutes of the region such as Agricultural Institute Osijek, Croatia will be discussed. These advantages include i) simultaneous education, training and employment of young researchers via "scientific novice" program of the Croatian government intending to rapidly develop a pool of diverse, high-quality plant researchers and breeders, and ii) cooperation with public agricultural systems abroad aiming to optimally allocate genetic, production, and human resources.

POSTERS
Session I
Genetic Resources

1.1 Agronomic characteristics and protein content of native maize (*Zea mays* L.) growth in Kosovo

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Maize (*Zea mays* L) is an important food crop and has as a crop of economic importance for our country. The objective of this research were; to determine the grain yield (GY), protein content (PC) and to characterize the current Kosovar landraces of maize for morphological, agronomic characteristics and to compare the genetic variability between their. The experimental design was a randomized complete block (RCB) with three replicates, while the plot sizes were 14m². The research was carried out in 20 native maize accessions were collected and 30 ears per accession were evaluated and characterized. All characters were measured in the field and laboratory at the normal harvest time. The quantitative and qualitative parameters were statistically studied through ANOVA. Kjeldahl method was used to determine proteins content (PC). Grain yields (GY) of 20 selected landraces were on wide range which varied from 47.31 till 109.95 g plant⁻¹. The overall mean μ of GY was 64.96 g plant⁻¹. The comparison of value μ among GY was -17.65 g plant⁻¹ or 27.17% and +44.99 g plant⁻¹ or 69.25%. The genetic variation was +62.64 g plant⁻¹ or 96.83%. The overall value μ for PC at all accession was 11.81%. The best local accession had PC of 13.02% while on low percentage of PC was 11.03%. Differences between accession of PC were +1.99 or 16.85%, statistically higher significant at level $LSDp=0.01$. These results suggest that some landraces are a potential source of alleles for improving grain yield and protein quality and these landraces would be useful for the development of new lines and hybrids.

1.2 Maize gene bank as a source of beneficial alleles

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The total of 41 accession from MRI maize gene bank was selected as the most drought tolerant, with good combining abilities (15 introduced inbred lines, 12 introduced populations and 14 local populations). This accessions were analysed for oil, protein and starch content using NIR technology. Sustainable variation was found for all of this traits. The range for oil was 2.8-5.5%, protein 9.6-15.2% and starch 66.6-71.6% of dry matter. The values for oil and starch are lower, and for protein higher than average for maize. That could be the consequence of the particular year of investigation, their drought tolerance etc. Existence of some specific proteins involved in increased drought tolerance will be a subject of further investigations. For oil and starch, introduced populations showed the highest average values, significantly higher than the other two groups of genotypes. Introduced lines, however, had significantly higher protein content. Considering all accessions, breeding for increased oil content would be efficient: expected genetic gain for 5% selection intensity is 57.2%, and for 20% selection intensity is 38.97%. For increasing protein values, breeding would not be so efficient, but still would be very beneficial (23.75% of expected gain for 5%, and 16.1% for 20% selection intensity). As far as starch is concerned, no breeding effort should be put on this material (2.82% of expected gain for 5%, and 1.92% for 20% selection intensity).

1.3 Parameters affecting photosynthetic activity of F1 grain sorghum hybrids obtained in different types of sterile cytoplasms

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One of mechanisms enabling sorghum adaptation to arid environment is high productivity of photosynthesis, which is conditioned by powerful leaf apparatus, specific anatomical features of sorghum leaves, and C4-photosynthetic pathway. Development of plant photosynthetic apparatus is known to be controlled by different genetic systems – nuclear genome and chloroplast genome. Therefore, screening new combinations of nuclear and chloroplast genome enables highly-efficient functioning of photosynthetic apparatus and opens new prospects for development of drought-resistant F1 sorghum hybrids. We studied the effects of new types of sterility-inducing cytoplasms on a number of parameters conditioning photosynthetic productivity of F1 sorghum hybrids – leaf area value, photosynthetic potential, and pure photosynthetic productivity. The isonuclear CMS-lines with Milo 10 genome in the A3, A4 and '9E' cytoplasms, and isonuclear CMS-lines with Pishchevoe 614 genome in the 'M35-1A' and '9E' cytoplasms were used as maternal parents. Cultivars Mercury and Pishchevoe 35 were used as pollen parents. It was found that the '9E' cytoplasm significantly increased the leaf area value and photosynthetic potential of the F1 hybrids contrary to the A3 cytoplasm, which significantly reduced these parameters. The '9E' cytoplasm also increased the pure photosynthetic productivity of the F1 hybrids at the 'tillering-heading' period, while the 'M35-1A' cytoplasm increased this parameter at the 'heading-complete maturity' period. Assuming previously discovered effects of these cytoplasms on the chlorophyll a and b and carotenoid content in the F1 hybrids (Kibalnik, Elkonin: Russ. Agric. Sci., 2009, V. 35: 20-23) it becomes clear that sterile cytoplasm type have significant impact on development of highly-productive F1 sorghum hybrid.

1.4 AMMI analysis as effective tool in development of maize (*Zea mays* L.) breeding concepts

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An additive main effect and multiplicative interaction (AMMI) model was used to analyze data of 6 ZP maize hybrids, widely grown in Serbia during last decade. The trial was assessed for grain yield in 16 locations representing main maize production area of Vojvodina, as well as smaller group of agro ecological sites in Central Serbia during 2009. The aim of this study was to answer the question, which breeding approach concerning GxE interaction was prevalent in MRI "Zemun Polje" and which should be. Is it breeding of plastic, widely adaptive hybrids or selection of genotypes adapted on particular micro environment? By criteria of GxE interaction two most similar sites were Salinac and Sombor. These two locations had practically identical rank of first four hybrids. Medium early maturity group hybrid, ZP 434 was winner of seven sites, with average yield surpassed over all average. Grain yield interval of variation of hybrids tested at all 16 sites was ranged from 2.604 tha⁻¹ to 12.58 tha⁻¹, while the over all average value was 7.391 tha⁻¹. AMMI model with two axes of interaction showed that the effect of genotype participates with 2.34% in the total sum squares of treatment, while the share of GxE interaction in the total sum of treatment squares was 5.58%.

1.5 Genetic variability among maize landraces (*Zea mays* L.) for ear and ear components

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With maize landrace 60 years ago in Kosova, were planted 100% of surfaces, while currently planting only 5 - 8%. The main objective of this study was evaluation of ten maize landraces: FAGB-01, FAGB-05, FAGB-07, FAGB-08, FAGB-12, FAGB-16, FAGB-18 and FAGB-19, in order to determine genetic variability among ear and ear components, for the farmer level of cultivation. The study has been carried during the 2009 and 2010 years, maize landraces are collected in different rural area, from 520 - 824 m of altitude. The structure was: Locality - L5, Maize landraces - ML2, Parameters - P5, Replication - R5, or experimental combination was: L5 x ML2 x P5 x R5) = 250. For analysis of ear components, the samples are taken after the full ripening, depending from the agro - ecological conditions of localities. The researched factors and parameters are: Localities, landraces, ear components and interaction between genotype and localities (G X L). Genetic variability was investigated for parameters: Ear length (EL), Number of rows per ear (NRE), Number of grains in row (NGR), Number of grains per ear (NGE) and Ear weight (EW). Interval of genetic variability on the average value for parameters were: EL (12.2 - 20.6 cm), NRE (9.2 -12.8), NGR (25.2 - 41.6), NGE (235.2 - 532.0) and WE (80.26 - 233.21 g). Statistical analyses were conducted using program MINITAB-16, and Excel program. Differences were highly significant for ear and ear components, for maize landraces (ML), localities (L) and for interaction between genotype and localities (G x L).

1.6 Chemical grain composition of maize landraces from the western Balkan region

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Oil, proteins and starch content was analysed in grains of maize landraces belonging to 18 agro-ecological groups from the western Balkan region that are conserved at Maize Research Institute gene bank. Three accessions per agro-ecological group were analysed. Oil content varied from 3.63% in Romanian flints to 4.73% in Montenegrin flints. The lowest average protein content was recorded in Derived flints landraces (10.75%), while the highest protein content was detected in landraces belonging to Montenegrin flints (14.05%). As expected, landraces of Montenegrin flints with the highest oil and protein contents had the lowest starch content (67.53%). The highest starch content (70.43%) was recorded in the grains of Derived flints, classifying them into high starch maize types. ANOVA did not show significance in variation in the average oil and starch contents among agro-ecological groups at both levels of significance ($p < 0.05$, $p < 0.01$). However, significant differences were established among agro-ecological groups in relation to the average protein content, at both levels of significance ($p < 0.05$, $p < 0.01$). The LSD method was applied to test the differences in oil, protein and starch contents between each group individually. Hierarchical clustering analysis was used for dendrogram construction. The dendrogram, whose landraces had similar chemical grain content, classified agro-ecological groups into appropriate clusters. Two principal subclusters were formed. The first subcluster grouped agro-ecological groups from the south-eastern area of the western Balkan (Montenegrin flints, Macedonian flints, Kosovo flinty flints). In the second subcluster the remaining agro-ecological groups were distributed in relation to geographical directions: continent-sea side and east-west.

1.7 Settling the potential of Portuguese maize germplasm for association studies

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Nowadays European consumers are more concern about the quality of their food and about agrobiodiversity erosion and unsustainability of agricultural ecosystems. Centuries of maize cultivation in Portugal have originated a very diverse germplasm with particular quality for food production (sourdough 'broa' bread) and with great value for breeding. Traditional Portuguese maize bread, with particular nutritional value, has high potential to become a popular specialty fighting health and sustainability public concerns. A diverse collection of inbred lines derived from traditional Portuguese germplasm has been developed and conserved at the Portuguese Plant Germplasm Bank (BPGV). In order to determine the potential of this collection for an association mapping approach aimed to identify the genetic basis of bread making quality, we studied its genetic diversity and population structure. 150 maize BPGV inbred lines were screened using 50 SSR markers uniformly distributed throughout the genome. A number of genetic diversity parameters was calculated (N_a , H_o , H_e and PIC) for the loci as well as the proportion-of-shared-alleles distance between pairs of lines. All showed the existence of large variability among inbreds. The Neighbor Joining tree depicted well the pedigree records of inbreds. Presently, linkage disequilibrium and the genetic structure of the collection are being analyzed. This information together with the phenotypic characterization of the lines will be crucial to select the most appropriate group of inbreds for the future association studies reducing the risk of false positives and maximizing the detection of significant marker-trait association.

1.8 Diversity studies using marker technology in maize breeding at ARC-GCI in South Africa

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Molecular markers are used extensively worldwide for detection of genetic relationships among germplasm entries in seed banks and breeding programs; determination of heterotic groups for hybrid breeding; identification of duplicates in germplasm banks as well as plant variety protection. Reif et. al. (2003) concluded that "SSR markers provide a powerful tool for grouping germplasm and are a valuable complementation to field trials for identifying groups with satisfactory heterotic response". The purpose of this study was firstly to evaluate the use of SSR markers to group South African inbred lines into heterotic groups. Secondly, it was to determine to what extent the expression of genetic distance between inbred lines are reflected in the actual yields of their hybrids in yield trials. Results of this study will be presented and discussed.

1.9 Evaluation of Romanian maize local landraces for increasing the efficiency of their use in breeding programs

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In the present it is noticed a main interest of plant genetic resources users for information accessing relating to characterization and evaluation descriptors. Maize genetic resources represented by local populations originating from different areas, represent important useful genes sources for species improving. Their exploitation it is possible through studies and comprehensive measures which may lead to the maintenance of biodiversity and increase its efficiency. The paper presents the results of characterization and evaluation of a total of 61 local landraces with cold test index >80%, selected from a total of 160 studied local landraces. These local landraces can be useful genes sources for maize breeding to low temperature, being a main trait for maize cultivation in wetter and colder areas from Romania. The study shows a high diversity for most of the plant morphological characterization descriptors, ear and kernel, physiological evaluation for low temperature resistance of the plantlets, precocity and biochemical evaluation for kernels quality. In order to obtain information, at intervariety level, for maize local landraces studied, molecular characterization was performed by RAPD method (random amplified polymorphic DNA). Many maize local populations with values of characterization and evaluation descriptors of real interest were emphasized. Utilization of these local landraces as starting material can lead to the identification of useful genes sources for improvement of important agronomic characters in maize (yield capacity, precocity, resistance to low temperatures, quality and genetic integrity).

1.10 Effects of multiple trait selection in maize (*Zea mays* L.) breeding

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The main goal of most maize breeding programs, apart from the type of source materials and breeding methods applied, is to obtain genotypes with high grain yield. As the yield is complex trait influenced by many factors and characterized by very low heritability, it is necessary to determine what are the secondary traits that largely determine the grain yield. Breeders usually wish to improve more than one trait in a breeding program. The traits may be correlated or independent from each other. In order to more reliably perform selection for yield and try to find adequate criteria to reduce the subjective estimation of property value, breeders simultaneously take into account the effects of multiple traits on yield. For this study six maize inbred lines originated from the same heterotic group are investigated throughout monitoring the effects of five traits that are considered very important in determining grain yield. Path coefficients (Edwards, 1979) as well as selection indices using couple of methods, SH index proposed by Smith and Hazel (1936), summation index (Mulamba and Mock, 1978) and Baker's standard deviation index are calculated. The experiment was set up in 2010, at two sites in two replications according to RCBD. Most of the calculated correlation coefficients of independent variables was significant in the rank of mathematical interdependence.

1.11 Pollen production of inbred maize lines in different sowing date and years

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The majority of maize varieties and hybrids are able to produce adequate quantity of viable pollen under various ecological circumstances, and pollen production cannot be considered as a limiting factor as far as yield is concerned (Duvick, 1997, Westgate et al., 2003). However, extreme abiotic stress factors may cause flowering asynchronism, reducing the chance of fertilization or may generate the production of less viable pollens in lower quantity. Plants are the most susceptible to decreased water supply right before and during the process of flowering. Water deprivation often results in the disorder of pollen production or even the complete lack of it (Hall et al. 1980, Herrero and Johnson 1980), or may delay the development of style. The response of genotypes depends on the extent of stress, and the inherited traits of the genotype under stress. Inbred lines are especially vulnerable to stress factors, which is manifested in pollen production and the viability of pollen grains as well. Due to the negative correlation between pollen production and grain yield, the number of new inbred lines with tassels of insufficient size, characteristics and unsatisfactory pollen production has increased, risking safe seed production. Plant breeders often select for small tassel size to reduce the dominance of tassels over cobs (Fisher et al. 1987). Westgate and Basetti (1991) found that inadequate pollen production caused by small tassel size reduced yield during top cross seed production. According to Ulibelarrea et al. (2002) little information is available on the pollen production of modern hybrids, while tassels are significantly smaller than in the past. Between 2002 and 2005, the pollen production of 19 inbred lines and 2 sister line crosses of Martonvásár were examined in three repetitions in split-split-plot arrangement in Martonvásár. Data were analysed using multifactorial analysis of variance in accordance with the instructions of Sváb (1967). Pollen was collected in pollen bags in each plot from 5 plants of the same flowering stage. The collecting of pollen was started on the day when the first anthers appeared on the tassels' main branches. Measurements were carried out until there was no measurable quantity of pollen in the bags any more. Pollen was cleaned of impurities (anthers, insects, etc.) using fine-meshed screen. Our aim was to determine the pollen producing ability of the inbred lines examined and the effect of the sowing date and year of production as an environmental factor on pollen production. The total pollen production of inbred lines as well as the duration of pollen production were analysed.

1.12 Genetic diversity among maize landraces assessed by SSR markers

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Maize landraces represent a valuable genetic resource that can be used in different breeding programs. Our objectives of this study were to develop genetic fingerprints for characterization of landraces and to estimate their genetic diversity. The genetic relationships among six landraces from ex-Yugoslavian territories (three belonging to Flinty dents agroecological group and three to Dents type of USA Corn Belt agroecological group) were analyzed using simple sequence repeat (SSR) markers on bulked DNA samples. Twenty-two highly informative primers amplified 208 alleles out of which 187 (89.9%) were polymorphic. Genetic distance values calculated using Nei and Li (1979) and were in the range from 0.48 (FD1 and FD3) to 0.68 (FD3 and FD2). Cluster analysis was performed using Unweighted pairgroup method (UPGMA). Cluster analysis grouped all three dent landraces in one cluster Two dent flint fell into the second cluster and one flint clustered with dents landraces. Combination of bulk SSR analysis and polyacrylamide gel electrophoresis showed that this approach could be used in characterization and genetic distance estimation of landraces from gene banks.

1.13 Interaction between different genes controlling endosperm development in maize

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In this report we present the results of a complementation test involving nine emp (empty pericarp) mutants of maize that represent single gene mutants, isolated as independent events. These mutants are embryo lethal at maturity and drastically reduced in their endosperm size. They can be subdivided in two major subgroups: those with a flat appearance of the kernel and those with a wrinkled pericarp. By crossing inter-se plants heterozygous for emp mutants, we identified those non-complementing (that means allelic) and those complementing (that means not allelic) in the F1 generation. Most results in the F1 were concordant to those obtained in the F2 generation with the exception of four cases where the F1 results suggest allelism (i.e. one gene) whereas those in the F2 segregation of two genes. This intriguing result seems to suggest an interaction between different emp mutants due to second site non-complementation (SSNC). In addition while scoring ears segregating for a single emp mutant, in different genetic backgrounds, we noticed that some mutant seeds exhibited a more abundant endosperm tissue and occasionally an embryonic axis. About 10% of these seeds germinate yielding slow growing seedlings. This observation could be explained by assuming that emp mutants introduced in different genetic backgrounds uncover a cryptic variability. This point needs to be further investigated but if confirmed, emp mutants could be used as a tool for the detection of genetic factors contributing to the amount of endosperm in the maize kernel to exploit in breeding programs.

Session III
Breeding for Specific Traits

3.1 Maize hybrids stability

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Hybrids being compared over varying environments usually rank differently. This made breeding companies allocate significant resources in hybrids testing programs. As it is practically impossible to create and to produce the most yielding hybrid for every single environment, all breeding companies tend to create and to produce seed of hybrids more or less widely adapted, hybrids that yields good in varying environments. Wide adaptation of hybrids, also, provides more stabile production over years, even at the same field. Presented results generally shows that most yielding hybrids are better adapted to good environments having b_i above 1 (high value of S). At the same time some of these hybrids have high values of S_{di} . Less yielding hybrids are better adapted to poor environment having b_i below 1 (small value of S), differing among themselves according value of S_{di} . Hybrid with the highest value of b_i had, at the same time highest value of S_{bi} , while hybrids with the low b_i generally tend to have low value of S_{bi} , too. Because , as mentioned, commercial hybrids generally are more or less widely adapted (at least if we have in mind target region, as precipitations were the main limiting factor for high yield obtaining), probably high values of S_{bi} can indicate more specific adaptation to good or poor environment depending on b_i . With a few exemptions, Eberhart-Russell and Finley-Wilkinson models gave similar results, comparable with the results of AMMI analysis.

3.2 The identification of bands related to yields and stability in maize hybrids

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Geneticists have been trying to explain adaptability and stability of a genotype in terms of a desirable combination of alleles expressed via epistasis. However, grain stability of a certain genotype can be a result of different factors, such as tolerance to drought, or to some important diseases and pests. Therefore, stability determined in one set of data is very often stability of a genotype to a prevalent stress factor in a moment of carrying out the experiment. Yield and yield stability of 15 maize hybrids were observed in 24 environments. The level of the interaction effect of studied maize hybrids was identified by the AMMI analysis, while a number of bands positively related to yield and stability of studied maize hybrids were identified by a genetic characterisation by RAPD markers. Bands positively related to yields were present to a greater extent in parents originating from the *BSSS* population, while bands positively related to stability were more present in parents originating from the *Lancaster* population.

3.3 Corn reddening – a step towards the solution

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Corn reddening (CR), observed for the first time in Serbia on a small area in the Banat region in 1957, has become a significant issue. Two serious outbreaks in the last decade (2002/2003 and 2010) considerably affected corn production not only in Banat, but also in many parts of the country. Controversial opinions on the biotic or abiotic nature of the occurrence seem to be overcome by discovery of likely phytoplasma aetiology. Whatever the cause, an interesting level of tolerance to CR was identified in a locally developed population. To assess the degree of tolerance to reddening among breeding materials, an intensive testing program has been conducted. Field trials were set up during 2009 and 2010 at two locations known to be epicentres of corn reddening. All materials were grouped in three pools: Local, BSSS and LSC. 120 S₃ families within each pool were randomly divided into five sets, with two replications within a set. The basic plot consisted of two rows with 100 plants planted at the density of 64,000 plants/ha. Significant differences were observed among the tested materials. The local pool showed the highest tolerance to corn reddening, followed by LSC and BSSS (4.3%, 15.1% and 26.3% plants with typical CR symptoms, respectively). Since the most frequently used combination in temperate zone (BSSS x LSC) could be pretty vulnerable in the years to come, some newly discovered gene sources could help in breeding for tolerance.

3.4 Relationship between seed deterioration and seed vigour parameters in maize (*Zea mays* L.) hybrids

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An experiment was set up on 10 maize (*Zea mays* L.) genotypes in 2010 in order to study the effect of various lengths of storage on the biological value of the seed and the correlation between seedling parameters and field grain yields. Samples taken from seed lots of the same genotypes stored for 1–11 years were examined in germination, vigour and cold tests. Apart from the usual parameters, a record was also made of the shoot fresh weight, root fresh weight and shoot length of 4-day-old seedlings. Laboratory analysis was followed by field trials, where emergence, plant development, flowering time, grain moisture at harvest, biotic stress sensitivity and grain yield were scored. Storage for five years or more led not only to a significant decline in seed vigour, but also to slow, protracted juvenile development, resulting in delayed silking and an increase in grain moisture at harvest. The mass and shoot length of seedlings subjected to stress decreased significantly with an increase in the storage period. The shoot mass of seedlings developing in a cold, oxygen-deficient environment was greater than the fresh root mass after any length of storage. A close correlation was found between the vigour of stressed seedlings (complex stressing vigour test: CSVT) and the grain yield (Y'), with an r^2 value of 0.805 (hybrids Mv 384 and Mv 265) and the regression equation: $Y' = 6.35 + 0.018 \text{ CSVT}$. An even closer correlation was obtained ($r^2 = 0.84$) between the fresh shoot weight of the seedlings (SW) and the grain yield (Y') in the case of hybrids Mv 343, Mv 384 and Mv 394 (regression equation: $Y' = 11.5 + 0.74 \text{ SW}$). There was a looser correlation ($r^2 = 0.37\text{--}0.55$) between the fresh root weight of the seedlings (RW) and the grain yield (Y'), with the exception of hybrid Mv 384 (regression equation: $Y' = 11.61 + 1.18 \text{ RW}$). It can be concluded that the field performance of the genotypes does not always depend on the length of storage. The initial vigour of the seed could be a more reliable indication of expected performance.

3.5 Ear rot severity, mycotoxin content and *Fusaria* species in maize hybrids grown in Poland

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Red and pink ear rot diseases are the most economically significant diseases of maize. Infection by *Fusarium* spp. results not only in yield reduction but also in contamination with mycotoxins. Compared to wheat, much less is known about the aggressiveness and mycotoxin production of *Fusarium* spp. on maize. Breeding for resistance is the best method to control this disease. *Fusarium graminearum* and *F. verticillioides* which are commonly connect with maize kernel samples might react differently to the same environment, thus the effect of genotype x environment interaction may multiply in infection trials. Because of this the resistance of the 14 -19 maize hybrids grown under field conditions in 3 localization in Poland, toxin content in the kernel samples collected from each hybrid separately and colonization of the kernel samples by *Fusarium* spp. were investigated during 2008 - 2010. Visual evaluation of ear rot severity was done at the beginning of October using 1 – 7 scale. The DON and fumonisins FB₁ – FB₃ content was analyzed using HPLC method in 2008 and using ELISA method in 2009 and 2010. The level of the disease in all localizations was very low, however the effect of the year and localization for the ear rot symptoms and toxin content was observed. Resistance data after kernel inoculation significantly related to the DON concentration. It was possible to observe a big differences in population of *Fusarium* spp. in the years 2008-2010. In 2008 *F. verticillioides*, *F. proliferatum* and *F. subglutinans* dominated. In 2009 and 2010 *F. graminearum* was much more common. Effect of genotype on population of *Fusarium* spp. was determined.

3.6 Responses of pedigree selection for ear and stalk rot resistance in F₂, F₃ and F₄ generations of maize

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Ear rots caused by *Fusarium graminearum* and *F. verticillioides* are some of the most economically significant diseases of maize occurring widely throughout maize growing regions of the world. Mycotoxins produced by those fungi represent a risk to human and animal health. Another very destructive disease in Central Europe is the stalk rot caused by *F. graminearum*. One way to control both diseases is to use resistant maize varieties. However, only a limited number of resistant sources were described. The major objective of this study was to develop improved maize genotypes with increased resistance to the ear and stalk rots caused by *F. graminearum*. Sixty seven maize populations were evaluated under field conditions. Plants from each population were selfed and inoculated with *F. graminearum* into the developing kernels. Ear rot resistance was scored after kernel inoculation using 1-7 scale. Stalk rot resistance was evaluated under natural infection, where *F. graminearum* prevails, using 1-9 scale. All genotypes rated lower than 3 for ear rot and lower than 5 for stalk rot were selected to continue the selfing procedure. Phenotypic variation of ear rot resistance was higher than that for stalk rot resistance, and both of them were important. Considering all populations, percentages selected in F₂, F₃ and F₄ were 41,9, 51,6 and 56,6, respectively. Significant differences within population were observed. The range of genotypes with moderate resistance within populations varied from 7,1% to 68,6% in F₂, from 14,3% to 71,3% in F₃, and from 16,7% to 74,4% in F₄.

3.7 The variations in grain chemical composition of some maize family triplets

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Sets of 9 hybrids and their parental inbreds from Maize Research Institute were grown with aim to determine the chemical composition of grain and possible influence of mother or father component on hybrid. The hybrids analyzed in this study were grown in split-plot experimental design with tree replications at Zemun Polje, during the summer of 2010. The grain yield, moisture content, crude protein, starch, oil, as well as, antioxidants like phytate, soluble phenolics and free sulfhydryl groups (PSH) were analyzed. The obtained data emphasized ZP 434, 555, 560 and 684 as hybrids with highest acquired grain yield, and relative low phytate content. Meanwhile, ZP 427 and 600 had the lowest grain yield, as well as relative high yield of their parental inbreds. The highest content of phenolics was also noticed in these two hybrids (up to 25 and 47% in relation to average of other hybrids). The parental inbreds of ZP 427 was characterized with inversely proportional content of oil, starch and PSH content. The high positive correlation was observed between yield and phenolic's content in hybrids, as well as yield and phytate content in inbreds, indicating importance of these two antioxidants in different category of maize grain. The seed moisture correlated in high degree with phytate and PSH, but only in grain of inbreds.

3.8 *In vitro* kafirin digestibility and selection for improved nutritive value of grain sorghum

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Improvement of nutritive value is one of the main goals of sorghum breeding. It is known that one of the reasons of relatively poor nutritive value of sorghum grain is resistance of its seed storage proteins (kafirins) to protease digestion. To explore genetic aspects of kafirin resistance to protease digestion we studied the F1 hybrids between the lines with different level of *in vitro* protein digestibility. In different hybrid combinations, different mode of inheritance of kafirin resistance was found. Relatively poor kafirin digestibility of the line Volzhskoe-4w was dominant in its hybrids with the line KVV-114, both in direct and reciprocal combinations. Kafirin digestibility level of hybrids between KVV-114 and Volzhskoe-615 differed in reciprocal combinations: the hybrids obtained using more digestible maternal line (A2 KVV-114) had significantly higher digestibility than the reciprocal ones obtained using relatively poor digestible maternal line (9E Volzhskoe-615). These data suggest importance of maternal genotype in determination of the F1 hybrid digestibility. Remarkably, the F1 hybrids between relatively highly digestible lines (A2 Karlikovoe beloie /Pishchevoe-614, A2 Karlikovoe beloie /KP-70, A2 KVV-97 /Pishchevoe-614) had significantly lower digestibility than parental lines except the M35-1A Karlikovoe beloie /KVV-45 that maintained high level of kafirin digestibility characteristic to paternal line. Such result suggests that relatively high digestibility of KVV-45 may be controlled by any dominant gene(s) contrary to recessive gene(s) in other lines. These data have importance for fundamental investigation of factors influencing sorghum seed storage protein digestibility (kafirin genes structure, structural organization of protein bodies) and for practical breeding.

3.9 Marker-trait associations for cold tolerance during juvenile development in sorghum

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Sorghum has high potentials for bioenergy production but is poorly adapted to low temperatures resulting in prolonged juvenile development. The adaptation of sorghum to tropical and subtropical highlands gives hint for certain genetic variation in cold tolerance. The goals of the present study are to identify fast growing sorghum genotypes and to detect marker-trait associations for temperature dependent growth rate parameters describing leaf growth, dry matter accumulation and chlorophyll content. A diversity set comprising 194 genotypes was tested in eight controlled environments with temperatures ranging from 9.4 to 20.8 °C. Leaf appearance, leaf area and dry weight were measured 7 days after sowing and at the end of the experiment. Chlorophyll content and Chlorophyll fluorescence were measured once in each environment. Mean genotype performance, growth rates and base temperatures were used for association studies. Sorghum lines were fingerprinted with 171 DArT and 31 SSR markers. The population structure was analyzed using Structure and association studies were performed with Tassel 2.1. Promising marker-trait associations for cold tolerance during juvenile development were detected on chromosomes 4 and 10. Future goals are fine mapping of QTL regions in order to identify stable markers enabling marker-assisted selection for cold tolerance.

3.10 Phenotypic and genetic analysis on the isonucleus lines in maize

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Isonucleus lines in a narrow sense indicate only the genetic material (line) of homeonucleo-hetero cytoplasm. Breeding for a isonucleus lines, has not only provided a new model of investigation into the genetics of cytoplasmic and nuclear genes and nucleoplasmic genetic interactions, but also has provided a fresh channel for avoiding a single cytoplasmic resource in germplasm and has increased the diversity of germplasm resource in crop production. The objective of this study was to determine the effect of six cytoplasm sources in five inbred lines on morphological and quantitative characters. Five isonucleus lines of maize were used as experimental materials. The genotype of each five original lines (TC 209, TC 243, TC 221, TB 367, D105) was backcrossed nine times into six cytoplasm (A665, T291, T248, TC208, K1080, W633) other than its own. For comparing the five isonucleus lines, each of them was crossed with three testers (inbred lines). The analytic experiments indicated significant differences among the cytoplasm occurred for plant and ear heights, ear length, ear weight, grain yield and grain yield (starch, protein, fiber content). The analysis of variances has shown that the difference in grain yield, dry matter in grain at harvest and unbroken plants at harvest is significant among cytoplasmic sources.

3.11 Biochemical and phenotypic analysis of quality protein maize breeding germplasm

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Maize has poor nutritional value due to deficiency of two essential amino acids – tryptophan and lysine. Although recessive *opaque2* mutation significantly increases their content in the endosperm, incorporation of *opaque2* into high yielding cultivars was not commercially successful, because of its numerous agronomic and processing problems due to soft endosperm. Quality protein maize - QPM has lately been introduced as *opaque2* maize with improved endosperm hardness and agronomic traits, but mostly within tropical and subtropical germplasm. The ongoing project at Maize Research Institute includes improvement of *opaque2* lines and conversion of standard lines to QPM germplasm. Herein, we present the results of biochemical analysis for whole grain tryptophan content and QI - quality index on F5 and BC1F3 plants of QPM x *opaque2*, *opaque2* x QPM and F5 plants of standard lines x QPM crosses. Tryptophan content and QI in F5 and BC1F3 of crosses between QPM and *opaque2* lines were over the threshold values for QPM germplasm in 72% genotypes. In seven out of nine genotypes of standard lines x QPM crosses they were also over the threshold values. Phenotype evaluation for ten traits was also performed. Most of the analyzed traits did not differ significantly. Average yield per plant (g) was found to be the highest in the progeny of *opaque2* x QPM crosses (314.3 g in BC1F3) and the lowest in standard lines x QPM progeny (195.9 g). The results will contribute to the choice of genotypes for further selection in breeding QPM.

3.12 A practical and robust bio-assay to screen maize varieties for resistance to *Rhizoctonia solani* AG 2-2 IIIB

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Rhizoctonia solani AG2-2IIIB has caused increasing problems in the culture of maize in Western Europe, especially when the crop is grown in rotation with beets, which is also highly susceptible to isolates of this anastomosis group. Fungicide treatments are either uneconomical or undesired and soil moisture content is not easily managed for field crops. In cases where crop rotations cannot be altered, disease resistance becomes the principal strategy to mitigate the problem. But only limited knowledge exists on the resistance level of the different commercial varieties. Our aim was to develop a practical and robust bioassay and apply it to test a set of varieties from the Belgian variety list. To develop the bioassay, different doses of ground or non-ground bran-based inoculum were evaluated together with different seeding depths and soil moisture levels. Consistent symptom development was obtained by using a controlled mixture of sand and potting soil, standardized inoculum preparation and application, seeding at a constant depth, controlled climate conditions and regimented watering during germination and seedling growth. A disease index was developed on a scale of 0 to 5. Root volume and the level of necrosis to the main and the adventitious roots were accounted for. Shoot weight was also used as an objective evaluation tool. Using the very disease-conducive conditions of the bioassay and two to three replicates consisting of up to 30 seeds each, 21 varieties from the Belgian variety list were tested for their level of resistance. Shoot weight varied between 10.9% and 44.3% of the non-inoculated controls. The average disease index was 3.4 and varied between 2.3 and 4.0, with significant differences being present between the varieties. A strong negative correlation ($R^2=0.76$) was observed between disease index and shoot weight. The disease resistance data will be made directly available to the growers via the national variety list. The bioassay is available to the commercial seed producers and can assist them when breeding for more resistant varieties.

3.13 The effect of seed priming on improvement of germination in corn (*Zea mays*) cultivars

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One of the most effects of salinity on plant growth, is the reduction in seed germination. The effect of Seed priming is the improvement of seed germination. In order to study the effect of seed priming on the improvement of seed germination, an experiment was conducted in factorial form using a completely randomized design with four replications. In this experiment two cultivars of corn (204, 704 single cros), four seed priming as: Distilled Water, KH₂PO₄, 1%, 2%, 3%. The seeds of corn, were placed in priming solution for 5, 15, 20 hours. The result shows that, there were significant difference among the variety of corn, priming solution, and the interaction between them. The best priming for percentage of germination was KH₂PO₄ 1%, for the root length was KH₂PO₄ 1%. There is no significant difference between priming and no priming for shoot length. More than 15 hours was not useful for improving indices germination. For 204 cultivar, the best priming for root length was KH₂PO₄ 2% in 204, and for 704 variety was Distilled water. KH₂PO₄ 1% was the best priming for improving shoot in all varieties.

3.14 The effect of seed priming on improvement of germination in sorghum (SPDF) cultivar

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Islamic Azad University Bojnourd Branch, Iran

One of the most effects of salinity on plant growth, is the reduction in seed germination. The effect of Seed priming is the improvement of seed germination. In order to study the effect of seed priming on the improvement of seed germination, an experiment was conducted in factorial form using a completely randomized design with four replications. In this experiment have done with SPDF cultivar of sorghum, four seed priming as: Distilled Water, KH₂PO₄ 1%, 2%, 3%. The seeds of sorghum, were placed in priming solution for 5, 15, 20 hours. The result shows that, there were significant difference among the priming time, priming solution, and the interaction between them. The best priming for percentage of germination was KH₂PO₄ 2%, for the root length was KH₂PO₄ 2% and 15 hours. The best priming for shoot length was KH₂PO₄ 2 %.

3.15 The effect of osmotic stress on proline content and peroxidase activity in seedlings of different maize genotypes

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Drought stress poses a major threat to crop production. Keeping in view the considerable demand for food, understanding about the biochemical and physiological basis of water stress tolerance in plants is vital to select and breed genotypes for improving crop water stress tolerance. Plants resort many adaptive strategies in response to abiotic stresses such as dehydration and excessive osmotic pressure. These adaptive mechanisms include changes in physiological and biochemical processes, among which, the accumulation of compatible solutes like proline and antioxidative enzymes such as class III peroxidases (POD). According to the field testing on 6.000 MRI gene bank accessions for drought tolerance under severe drought conditions (Egypt), as well as under moderate conditions (Serbia and Macedonia), we chose different maize (*Zea mays* L.) genotypes including inbred lines, introduced and local landraces, considered as drought tolerant, to investigate osmotic stress induced by polyethylene glycol (PEG), as osmoticum. The response to PEG treatment of these genotypes was analyzed in respect to their root and shoot length, fresh and dry weight, as well as to proline content and peroxidase activities. Root and shoot development was less in all genotypes under PEG treatment. Proline content increased in all genotypes, while peroxidase activity decreased and isoelectric focusing revealed different isoforms in PEG- treated comparing to the control plants for the most of tested genotypes.

3.16 Resistance of popcorn hybrids to *Fusarium* ear rot and fumonisins accumulation in grain

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Fusarium verticillioides is one of the most prevalent ear rotting pathogens of popcorn in Croatia, causing accumulation of fumonisins in grain. Resistance breeding and cultivation of less susceptible genotypes is the only efficient control strategy to prevent mycotoxin contamination. The objectives of this study were to evaluate the resistance of popcorn hybrids to fusarium ear rot, to analyze fumonisins accumulation in grain and to determine the correlation between ear rot rating and fumonisins content. Nine popcorn hybrids were grown at location Osijek in 2009 and 2010. Resistance to ear rot was evaluated in artificial infection with *F. verticillioides* according to method of Reid *et al.* (1996). Fumonisin concentration was analyzed using commercially available enzyme immunoassays (Veratox test kits). The rating of fusarium ear rot across two years ranged from 0.4 to 24.2 % and fumonisins content ranged from 0.0 to 8.8 mg kg⁻¹. Significant genotype, environment and genotype by environment effects were found for both traits. Phenotypic correlations between ear rot rating and fumonisins content were moderate ($r=0.57$ and $r=0.68$ in 2009 and 2010, respectively). Based on the obtained significant differences among hybrids for fusarium ear rot severity and fumonisins content, the most susceptible genotypes should be discarded. For identification of resistant commercial popcorn hybrids with reduced fumonisins content, evaluation of ear rot severity as well as fumonisins content in multi-environment trials is required.

3.17 Resistance of maize hybrids and inbreds to the maize redness syndrome

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The maize redness syndrome (MRS) was registered for the first time in south Banat (Serbia) in the period from 1957 to late 1960s, and then it has been registered since 2002. This disease can reduced the maize grain yield up to 70-90%. In order to establish disease control measures and stable maize production, resistance of 18 F₁ hybrids and 34 inbred lines of maize were observed under different agroecological conditions (Zemun Polje, Dobrica and Samoš). The trial was set up with three replications and 30 plants per replication. The disease severe intensity (DSI) was estimated at the milk stage on the basis of characteristic symptoms that were of special diagnostic importance - reddish purple colour of leaves that even spread over the midrib, exposed stalk parts, sheath and husk. These changes occurred rapidly, hence in a week or two the whole crop got red in colour, and then plants dried. Kernels in stricken plants were wilted and to a greater or smaller extent filled and shrivelled, and in some cases they were not even formed. Although MRS was established in all observed maize genotypes, the disease intensities differed. Six F₁ hybrids were more resistant than parental averages. According to the average of DSI in three locations, the most resistant genotypes were two hybrids (ZP836, DSI = 15.6%**; ZP 580, DSI = 16.50%***) and two inbreds (KL107, DSI = 22.17%*; PL186: DSI = 22.2%*). These results indicate that a special attention should be paid to control of this disease in maize breeding for resistance to MRS.

3.18 Electrophoretic analyses of zeins in breeding for quality protein maize genotypes

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Zeins are the major type of protein in maize kernel accounting for 50-60% of the total seed protein. They are deficient in nutritionally important amino acids, such as lysine and tryptophan, thus causing poor protein quality of traditional maize varieties. Although opaque-2 maize cultivars, low in zeins content, are twice nutritious as standard maize, they have not been accepted due to their soft, chalky endosperm texture which result in lower yield. Conversion of opaque-2 maize into varieties with high nutritional quality, conventional hardness and traditional appearance led to development of Quality Protein Maize (QPM). This has been done mostly within tropical and subtropical germplasm. The ongoing project at Maize Research Institute includes improvement of *opaque2* lines and conversion of standard lines to QPM germplasm. The objective of this work is to characterize qualitative and quantitative differences in zein content in normal, opaque-2 and QPM genotypes, using isoelectric focusing (IEF-PAGE) and sodium dodecyl sulphate-polyacrilamide gel electrophoresis (SDS-PAGE). Preliminary results reveal evident changes in major zeins components (α , β and γ) among the analyzed genotypes. Tested QPM germplasm from last performed cycle of selection contains relatively high amounts of γ -zein in comparison to normal and opaque-2 genotypes. The same QPM genotypes show significantly reduced amounts of α and β zein from normal and opaque-2 maize germplasm. While the reasons and significance of these findings are still to be discussed, zeins electrophoretic analyses, together with other biochemical and phenotypic evaluations, point out the successful conversion of opaque-2 and normal genotypes to QPM germplasm.

3.19 QTL analysis of maize ear inflorescence in a fasciation contrasting inbreds cross PB260 x PB266

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Maize ear inflorescence architecture is intrinsically important to grain yield components. Thus a thorough knowledge of the genes affecting ear inflorescence architecture and their interactions may lead to better yield modelling. Maize ear fasciation, characterized by abnormal flatten ears with higher kernel row number, is widely present in Portuguese maize landraces showing quantitative variation. To elucidate the genetic basis of ear inflorescence architecture traits related with fasciation, F_{2:3} families were developed from a fasciation contrasting cross (PB260-nofasciation x PB266-fasciation). F₃ lines were evaluated for 28 ear architecture traits plus yield, in two Portuguese environments (Caldeirão and Montemor-o-Velho). Highly significant correlations were found between fasciation and other traits. Fasciation broad-sense heritability was 0.73. Principal Component Analysis was performed to isolate the factors that defined overall variation in maize ear architecture. First three principal components accounted for 71 to 73% of variation. In order to map quantitative trait loci (QTL) for these traits, a genetic linkage map was constructed in F₂ population. Overall, 64 QTLs for individual ear architecture traits (38 in Montemor and 26 in Caldeirão), explaining individually 8.7 to 20.0% of the phenotypic variance, and 12 QTLs for the first three PCs (6 in Montemor and 6 in Caldeirão), explaining individually 10.7 to 22.4% of the phenotypic variance, were detected. 12 QTLs were conserved through environments. This analysis provided initial insight into the genetic basis of quantitative variation of fasciation related ear architecture traits. Future studies should focus on fine mapping of the identified genome regions.

3.20 Possibilities of resistant starch production from normal dent maize grain

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Resistant starch (RS) is the portion of starch that is not digested in small intestine, but is fermented by microflora in the colon. RS provides more health benefits than do dietary fibers. RS has been reported to have potential as a unique ingredient that can yield high-quality foods. The objective of this study was to investigate the possibilities of RS production from normal dent maize genotype by starch autoclaving and debranching with pullulanase (PromozymeBrewQ). Advantages of debranching over mild acid hydrolysis include shorter processing time, better processing control and higher RS yields. Starch from the maize grain was isolated by applying a 100-g laboratory maize wet-milling procedure. It was found that there were not significant differences in physico-chemical characteristics between starches of different dent ZP maize genotypes therefore we selected the genotype ZP 434 for RS preparation because it ranks among top hybrids produced in Serbia. Autoclaving at 120°C (30 min) increased the RS contents of all samples, whereas freezing at -20°C did not show obvious effect on the RS contents. The highest RS yield in autoclaved starch samples was 7.0% after three autoclaving-cooling cycles. After pullulanase debranching at 50°C and retrogradation at 4°C RS yields ranged from 10.2% to 25.5% in all samples (depending of hydrolysis time). The debranching effect of pullulanase on gelatinised maize starch to help RS formation is obvious and more effective than autoclaving, indicating this treatment with the enzyme is applicable in RS preparation to increase RS yield.

3.21 Adaptability and yield stability of maize hybrids (*Zea mays*) in different environments of Iran using AMMI and SREG GGE biplot analysis

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Introduction of superior genotype among diverse evaluated genotypes is specific objective of multi-environment trials (MET). Moreover, finding the corresponding genotype with its ideal production environment is another key point when there is significant genotype-by-environment interaction (GEI). The objective of this study was to assess the effect of GGE, on grain yield of 12 maize hybrids (*zea mays*) in 12 different research stations during two years of Iran. GGE biplot was used to visualize adaptation and yield stability of genotypes. A site regression (SREG) analysis was also taken to identify stable genotypes. The biplot illustrated 62.16% of treatments SS, with 2.25% due to G SS, 84.34% due to E SS, and 6.18% due to IPC1 and IPC2 SS. The first four bilinear AMMI model terms were highly significant ($p < 0.01$) and of which the first two IPCs explained 46% of the GEI variation. IPC1 and main effect, yield mean, totally explained 90.12% of the treatment variation. According to the AMMI and SREG GGE biplot models, five mega environments detected but no single variety showed superior performance in all the environments. The genotype SC726 was overall winner in combining high yield with relatively less variable yield across environments. The highest yielding environment among the 23 environments was Darab in 2003, which used to evaluate the genotypes and the performance of top two yielding genotypes, SC726 and SC647, compared in GGE biplot. Application of AMMI and GGE biplots facilitated visual comparison and identification superior genotypes for each target set of environments.

3.22 Field evaluation of sorghum landraces introgressed with quantitative trait loci for resistance to the parasitic weed *Striga hermonthica*

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The parasitic plant *Striga hermonthica* is a serious biologic constraint in the production of sorghum [*Sorghum bicolor* (L.) Moench] and other cereals in sub-Saharan Africa, where it causes substantial yield losses. *Striga*-resistant and well adapted sorghum varieties are seen as an important control option. However, conventional resistance breeding is hampered by a strong environmental influence on the development of the pathogen and a low heritability within field trials. If proven to be successful, marker-assisted selection (MAS) for genomic regions of *striga* resistance could help overcome these limitations in resistance breeding. In previous projects Quantitative Trait Loci (QTL) of the resistant Indian Durra-race sorghum line N13 were mapped and subsequently introgressed into two Guinea-race farmer-preferred sorghum varieties from Mali, West Africa, using a MAS-supported backcrossing scheme. Here, we report the field-evaluation of a selection of the introgression backcross-two lines (BC2S3) under natural and artificial *striga* infestation at three sites in Mali in 2009 and 2010. Preliminary analyses of the data show a resistance of the best sorghum lines equal to or exceeding the resistance of the donor parent N13. However, resistance was not always directly correlated to the number of introgressed QTL. The grain yield of the BC2-lines was on average inferior to that of the recurrent parents. A strong environmental influence on the *striga* resistance of lines was observed within and between trial sites. The effect of the introgressed QTL and their usefulness in breeding programs will be discussed.

3.23 QTLs for drought tolerance in maize: yield, yield components and ASI

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Drought is one of the most important limiting factors in maize production. Improving drought tolerance is therefore of outmost importance for breeding. Achieving this goal is a big challenge due to the variability in the timing, intensity and duration of drought, the genetic complexity of drought tolerance and large genotype by environment interactions. Low yield heritability under drought conditions makes selection for this trait inefficient. Yield components show greater heritability so it is possible to select for them under stressed conditions. Selection for secondary traits which are correlated with grain yield and have relatively high heritability may increase selection efficiency. In the experiment presented herein, 116 F3 families of DTP79xB73 cross were evaluated in the field trials under drought to map QTLs underlying yield, yield components and ASI. Phenotypic correlations calculated using Pearson's coefficients were high and significant. QTL detection was performed using composite interval mapping option in WinQTL Cartographer 2.5. A total of 30 QTLs were detected: five for yield, 22 for yield components and three for ASI. Percent of phenotypic variability determined for the identified QTLs for all the traits was in the range from 0.1% to 45.41%. Mapping analysis identified genomic regions associated with two and tree traits in a manner that was consistent with phenotypic correlation among traits, supporting either pleiotropy or tight linkage among QTLs. In this context, QTLs identified in our work and further research will help us elucidate genetic basis of drought tolerance and enable more effective breeding for drought tolerant maize hybrids.

3.24 Improving the resistance of maize inbred lines to diseases

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Fusarium graminearum and other fungi of the genus *Fusarium* are major causes of stalk and ear rot of maize in Croatia. *Colletotrichum graminicola* causing leaf blight and antracnose stalk rot is an increasingly spreading pathogen in narrow crop rotation and monoculture. Breeding for resistance is the most effective way of maize disease control. The purpose of this study was to assess how the program of improving line resistance to diseases was successful considering their combining ability for yield. The lines were tested under artificial inoculation in nurseries from 2007 to 2009. Testing combining ability was made in small scale trials in Rugvica in 2010. Eight hybrid combinations with new lines stood out with higher or significantly higher grain yield compared to the official standard. Newly developed inbred lines expressed improved degree of resistance to the diseases the breeding was focused to. Lines Bc 61014-3 and Bc 61473-14 were significantly more resistant to anthracnose stalk rot. Significantly lower percentage of rotted plants was found in lines Bc 62742-13, Bc 65741-26 and Bc 65073-3. Line Bc 63473-13 is a result of improved resistance to *Gibberella* ear rot. Resistance to targeted diseases was successfully improved by using methods of artificial inoculation in the breeding process. No negative effect on combining ability for yield was recorded.

3.25 Towards a sorghum ideotype with optimized cell wall degradability: insights from degradation kinetics, histological, biochemical, and pre-treatment analyses

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In addition to being the fifth cereal in terms of grain production, the interest of sorghum as a feed and energy crop is increasingly underlined. Selection of sorghum genotypes for fodder and energy production relies on an in-depth understanding of stem tissue organization and cell wall characteristics variability. Although histological analyses of two genotypes harboring contrasting stem diameter revealed similar tissue organizations and cell sizes, their kinetics of biomass degradation when treated with enzymatic cocktails were different, the largest stem diameter genotype presenting a faster degradation with a lower percentage of monosaccharide solubilised, suggesting differences in soluble sugar content and cell wall polymer content and accessibility. According to these results we then focus our attention on the cell wall composition. From a previous characterization of 410 sorghum accessions using near infrared reflectance spectroscopy, a panel of 13 genotypes maximizing the diversity of cell wall properties were subjected to FTIR analyses, klason lignin and *p*-hydroxycinnamic acids contents measurement, and determination of lignin monomeric composition. In parallel, saccharification efficiency of the cell walls was evaluated. These analyses revealed a significant genetic variability of cell wall saccharification yield which has been associated with the variability of the lignin content in the cell wall, the S monomers content in the uncondensed lignin part and the concentration in esterified *p*-coumaric acid. Finally, addition of an Alkali pre-treatment before the enzymatic treatment showed significant improvement of cell wall saccharification yield but contrasting responses of the genotypes analyzed in terms of final yield and degree of improvement.

3.26 Per se and test-cross performance of the M3S maize population for grain yield and stalk rot resistance after three cycles of recurrent selection

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Recurrent selection has been used for improvement of grain yield and disease resistance in genetically broad based populations. Maksimir 3 Synthetic (M3S) maize population was created at the Faculty of Agriculture University of Zagreb by intercrossing 12 domestic inbred lines showing good combining ability with both BSSS and Lancaster testers. The population has been subjected to three cycles of recurrent selection to improve grain yield and stalk lodging resistance. In the first two cycles selfed progeny selection were used and in the third cycle S1 and full-sib (FS) selection methods were conducted separately. The original population C0 and four advanced cycle populations C1, C2, C3-S1 and C3-FS, per se and their test crosses with inbred lines B73 and Mo17, were evaluated over three years under natural conditions (NC) and artificial stalk infection (AI) with *Colletotrichum graminicola* (Ces.) G.W. Wils. In the population per se grain yield increased 10% after two cycles of selection under NC. After the third cycle there was no further improvement in grain yield. Under AI an increase of grain yield was observed only in the third cycle for the FS selection. In the case of test-crosses an increasing trend for grain yield was observed only in crosses with Mo17 under NC. A significant decrease of stalk rot incidence from C0 to C3 was observed for both NC and AI with a higher response for C3-FS than for C3-S1. A significant decrease in stalk rot incidence in test-crosses occurred in the first cycle of selection only.

3.27 Assessment of morpho-physiological traits of sixteen maize (*Zea mays* L.) genotypes under salinity conditions and yield estimation using Artificial Neural Network (ANN)

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To investigate the effect of salinity stress and evaluate morpho-physiological features, sixteen different maize genotypes were chosen. Twelve morpho-physiological factors in adult plant were recorded. A factorial experiment was conducted using a completely randomized design with four replicates. The highest and the lowest shoot dry weight amount were related to SC370 and SC704, respectively. Leaf density of SC647 was the highest. DC370 had the lowest Na absorption amount and the highest K absorption. Regarding to the salinity tolerance and yield amount DC370 and SC647 introduce as candidate genotypes. The ANN is one of the most important soft computing techniques to predict output such as yield amount. This technique was used for modeling the correlation between crop yield and twelve yield components of maize. The ANN performed on all data and showed 88% correlation between actual and predicted yield data. Among the various ANN structures, a model of good performance was produced by 13–10–5–1 structure with a training algorithm of back-propagation and hyperbolic tangent transfer function in the hidden and output layers. The model was able to predict the maize yield data with a RMSE and R² value of 0.0029 and 0.77, respectively. R² value measures the scattering around line. The results showed that the highest bias in predicting model relate to higher salinity level (S5). However, the results of ANN showed that the technique can be successfully applied to predict yield even though in stress condition in terms of physiological and morphological traits.

3.28 Quantitative trait loci for metal accumulation into maize leaf

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Maize as a major crop has been investigated for decades regarding metal accumulation, but not in the context of leaf ionome to identify putative genetic factors participating in the control of metal accumulation. Our objectives were to analyze variation for copper (Cu), iron (Fe) potassium (K), manganese (Mn), magnesium (Mg), strontium (Sr) concentrations in leaves of a maize mapping population, and to detect and to determine the effects of QTL associated with the metal concentrations. Ear-leaf samples at the beginning of the silking stage was taken for elemental analysis (ICP-OES) of 290 F₄ lines of a biparental population (B84 x Os6-2) grown in field trials in Croatia. The population and parents differed significantly for Cu, Fe, K, Mg, Mn and Sr concentrations. The population was mapped using sets of 56 SNP and 65 SSR polymorphic markers. Eleven significant QTLs were detected for all six metal concentrations. Three of them, QTLs for Cu, Fe and Mg were colocalized on chromosome 5 in the region of *ys1* gene. Significant dominant effect of these QTLs supports the involvement of *ys1* in accumulations of these metals. Some QTLs had no obvious candidate genes offering the possibility of identifying unknown genes that affect metal accumulation.

3.29 Genetic similarity of sweet corn inbred lines in correlation with heterosis

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The narrowness of the sweet corn genetic base limited to the great extent its genetic improvement. This is associated also to the fact that sweet corn does not have well defined heterotic groups such as those important in field corn breeding. Therefore application of marker assisted selection (MAS) becomes a necessity in sweet corn breeding programs. The objective of this study was to determine genetic similarity (GS) of six sweet corn inbred lines selected in Maize Research Institute Zemun Polje, based on SSR markers and to compare it with the data of specific combining ability (SCA) and heterosis for fresh ear yield, obtained in a diallel study. SSR markers showed that all genotypes had specific genetic pattern. Estimates of GS varied from 0.381 (ZPLsu6 – ZPLsu2) up to 0.744 (ZPLsu6 – ZPLsu5). Cluster analysis and PCA showed clear groupings of inbreds into two subclusters, with inbred ZPLsu3 less related to the others. Results of diallel analysis of these inbreds showed that data of heterosis and SCA were in concurrence with the data of GS based on SSR markers. Hybrid combinations with higher estimates of SCA and heterosis expressed less genetic similarity with each other (such in hybrids ZPLsu6 x ZPLsu2; ZPLsu2x ZPLsu1 and ZPLsu5 x ZPLsu2) and vice versa. That was confirmed by rank correlation coefficient, whose estimates were negative and in almost all cases significant, indicating that more similar inbreds produced less expressed effects of heterosis and SCA in their hybrid combinations.

3.30 Genetic divergence between QPM inbred lines assessed by SSR markers

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Quality protein maize (QPM) contains *opaque-2* gene along with modifiers for kernel hardness. QPM is maize with high nutritive value of endosperm protein, with substantially higher content of two essential amino acids - lysine and tryptophan, and with good agronomical performances. Although QPM was developed primarily for utilization in the regions where maize is the main staple food (tropical and subtropical regions), it also has many advantages for food and feed production and consumption in other parts of the world. The ongoing project at Maize Research Institute aims at breeding QPM hybrids for temperate climate regions. Herein, genetic diversity estimation between 24 QPM inbred lines (adapted to temperate regions) is presented. These lines were analyzed with 36 SSR markers (105 bands). Statistical analysis was performed using NTSYSpc 2.1 program package. On average, genetic similarities calculated using Jaccards coefficient, were low (< 0.5). The highest genetic similarity was 0.84, and the lowest 0.17. Cluster analysis formed a dendrogram with two clusters that grouped the lines mainly according to their origins. The QPM lines were also submitted to kernel modification and biochemical analyses. Although defined as QPM, three lines had poorly modified kernels, while two lines had low tryptophan content and quality index. Combination of data on genetic similarities, cluster analysis, kernel modifications, tryptophan content and quality index, as well as phenotypic traits, can be used for selection of parental lines for breeding QPM hybrids. Low genetic similarities, different clustering, well modified kernels, high tryptophan content and quality index are recommended.

3.31 Genetic characterization of senegalese varieties of sorghum [*Sorghum bicolor* (L.) Moench] by microsatellite markers: identification of ideotypes

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Cultivar resistance is respectful of the environment and appeared as the most efficient way to fight fungal infection of the grains of sorghum grown in semi-arid conditions of the Sahel. This work aims at improving sorghum cultivars from Senegal through a program of markers' assisted selection. A set of 25 microsatellite markers is selected from 48 pre-selected ones and are used to characterize the local cultivars CE180-33, F2-20, CE145-66, CSM-63, CE151-262 and 93B-1057. The analyses are in progress. The results will help identify ideotypes having the most efficient drought tolerance and fungal resistance, useful in crops breeding.

3.32 Effects of cytoplasmic male sterility and xenia on seed quality of two ZP maize hybrids

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Growing a sterile hybrid as a female component and a fertile hybrid as a pollinator is a recent approach in maize cultivation that has been observed and referred to as the *Plus-hybrid* system. The *Plus-hybrid* effect on hybrids was studied with the aim to theoretically establish whether cytoplasmic male sterility (*cms*) and xenia affected traits that determined maize seed quality. In hybrids developed on the sterile basis, the female component has *cms*, while a male component is a fertility restorer, and therefore *cms* and xenia affect the commercial hybrid seed production. Two sterile hybrids and their fertile counterparts as female components (ZP 1 and ZP 2) and five fertile hybrid-pollinators were selected for the study. Moreover individual and *Plus-hybrid* effect of *cms* and xenia were observed. Germination, as a parameter of seed quality, was tested: 1) under optimum conditions (maximum germination potential); 2) under cold conditions as a parameter of vigour (cold test); 3) germination after seed exposure to conditions of accelerating ageing (seed longevity). Tests showed that observed factors *cms* and xenia and *Plus-hybrid* effect did not significantly affect standard germination, nor the length of the radicle and plumule. However, the values of seed germination under cold conditions and after seed exposure to conditions of accelerating ageing significantly differed between hybrids ZP 1 and ZP 2, but they were not expressed over effects of *cms* or xenia. It can be concluded that *cms* and xenia did not affect seed quality.

3.33 Trends in productive abilities of maize hybrids from different FAO groups

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Ten years data (2001-2010) from official maize hybrids testing in IASAS and preliminary (ecological) trials of Maize Research Institute (MRI), carried out in different locations are compared. Besides the actions of many occasional factors – diversity of environments and great number of hybrids tested from 5 FAO groups (200-600) every year, common tendency of both groups of trials are observed. Grain yield (t/ha) has a relative maximum in semi-late hybrids (FAO 500-600). Closer to them are earlier groups – FAO 300-400 and 400-500. Correlations between grain yield and vegetation period like FAO groups are not significant and their regressions are not linear. Grain moisture at harvesting (%) and vegetation period are strongly positive and regressions are linear for both of trials. For the period of investigation a progress of reduction of that trait was observed. Best combinations of relatively high grain yield and low grain moisture were pointed out for early hybrids (FAO 300-400) by performance index (Pi) values. Relatively good combinations of grain yield and stability were observed for FAO 500-600 hybrids, represented by their general adaptation index ($x_i - b_i$). IASAS trials, like final step of maize hybrids testing including bulgarian and foreign varieties with the same checks outyielded MRI trials as average levels by FAO groups, but overcoming the correlation high yield – late maturity, better p_i values for earlier genotypes and their adaptation are very similar in both trials groups like general tendency. These results confirm the “post green revolution” trends in maize breeding discussed by Duvick and Gassman (1999).

3.34 Productivity levels of maize hybrids with different portion of CMS

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Two maize hybrids – Kn 619 and Kn 517 were tested 2 years (2009-2010) at two plant densities including 3 portions of CMS (“mixed scheme”) – 33%, 50% and 66% of C and S type, respectively for both varieties. Regardless of xenia effect and GxE interaction, the variants with 50% CMS portion surpassed grain yield of the fertile checks – from 8 to 11% respectively for both hybrids. As an average level from all blended variants the out yielding is 3-4% for Kn 517 and Kn 619, compared even with their sterile checks (100% CMS). The experiment was enlarged with 3 other hybrids in 2010. The mean results from first year investigation are from 6,4% to 11,4% higher grain yield for the entries with 66% CMS portion compared to their fertile checks. The preliminary conclusions of that study are in a good accordance with other authors. So called “plus hybrids” system after Weingarthner et al. (2002) is very promising for farmers. The seed production of Kn 619 in 2010 was done in this way – with 66% share of CMS.

3.35 Relative efficiency of selection for maize grain yield under random drought stress compared to managed drought stress and well-watered conditions

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For breeding drought tolerant maize, selection for grain yield performance under drought stress can be conducted directly under random drought stress (rDS), or indirectly either under managed drought stress (mDS) or under well-watered conditions (WW). While rDS can occur at any plant developmental stage in the rainy season, drought stress is induced at anthesis in mDS trials conducted in the dry season. In this study we tested whether selection based on mean grain yield across WW or mDS environments is effective in improving grain yield of maize plants grown under rDS. In total 502 maize single cross hybrids of diverse maturity were evaluated in 548 trials in 17 countries in Africa from 2001 to 2009. Each experiment comprised an alpha lattice design with three field replicates and included 24 to 65 genotypes. The experiments were categorized into mDS, rDS and WW, on the basis of the irrigation management and the mean grain yield. The relative efficiency of indirect selection (RE) under WW conditions was on average higher than that of direct selection under rDS. Further, the RE under mDS was always lower compared to that of WW conditions. This was due to the lower heritability of grain yield under mDS compared to that of WW conditions and to a low genetic correlation found between rDS and mDS compared to that between rDS and WW conditions. These results suggest that breeders could select genotypes adapted to both rDS and WW conditions even when only WW conditions are considered.

3.36 Identification of drought tolerance maize populations among the French Maize network of genetic resource collection

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Maize demands water in summer when the availability of water is low. The objective of this study was to identify potential sources of tolerance to water deficit among the French INRA-PROMAIS Maize network collection (1400 temperate populations). One hundred forty eight populations selected for their phenotype on precocity, anthesis silking interval (ASI), combining ability, lodging tolerance, were evaluated under well watered and stress conditions in 2008. Stress conditions were applied between 15 days before flowering to 7 days after flowering. The best 50 populations were repeated in 2009 under similar conditions, based on selection index identifying populations with low ASI under stressed conditions, low decrease values for yield, number of grains, and fertility. In 2009, the evolution of leaf area was estimated as a function of time (rhythm of leaf appearance, length and width of leaves, evolution of senescence) in order to study the impact of drought on growth. The coefficient of correlation between ASI and yield was significant under stress conditions. No correlation was found between decrease of yield (average 67%) and leaf area (average 15%) which is consistent with a stress occurring just before the beginning of flowering (controlled by neutron probes). Populations exhibiting a low yield decrease and low ASI were often improved populations (synthetics, composites). However, some landraces (from Bulgaria, Algeria) showed low LAI decrease and low yield decrease. The results confirm the interest of genetic resources as source of variability to further improve maize varieties, and the improvement of maize already done with selection.

3.37 Genotype and year effect on morphology, yield and digestibility of silage maize hybrids

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Silage maize is the most important basic forage of ruminants. In the recent past silage maize hybrids were classified according to the fresh and dry matter yield and the proportion of the ear in the dry matter. Today the metabolizable energy yield and the digestibility of the forage is also an important aspect when ranking hybrids. The aim of our work was a detailed examination of the silage maize hybrids bred in Martonvásár. Eight hybrids with different type and different maturity periods were tested in three years (2002-2004) in Martonvásár. Morphological characteristics, yield, the proportion of the different plant parts in the dry matter, the chemical composition of the whole plant and the different parts, and digestibility were measured. It was concluded that yield was mainly affected by the year, while morphological traits, chemical composition and digestibility by the genotype. The year had the greatest influence on the dry matter yield per plant, and the least influence on the ratio of ear height to plant height. In case of chemical composition and digestibility the differences between the genotypes were greater than between the years. The morphological traits influenced digestibility less than the chemical composition. There were big differences in the chemical composition of the different plant parts. The experimental data were used to calculate the digestible dry matter yield of the hybrids. This is a suitable method of estimating the real value of the silage maize varieties. Hybrids with high yields but poor digestibility produced lower digestible dry matter yields per hectare than those with lower yields but better digestibility.

3.38 Effect of different germplasm on the content of antioxidant compounds in maize grain

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Because maize is a relevant food source, the quantification of the grain constituents with a health and nutritional role is important for the best exploitation of the different genotypes. The aim of this study was to determine grain antioxidant compounds content in 10 maize single cross-hybrids, divided in two groups respectively to the origin of their female components, Lancaster i.e. BSSS. The content of polyphenols, carotenoids and tocopherols, as well as, their antioxidant activities were determined in whole grain of maize hybrids grown at two locations. A female inbred originated from the Lancaster population had a higher effect on the polyphenols content in grain of hybrids. The content of total phenols, tannins and flavonoids ranged from 1.96 to 2.60 mg/g CE, 0.542 to 0.834 mg/g CE and 0.056 to 0.071 mg/g CE, respectively, in the hybrids grown at location of Zemun Polje and from 1.87 to 2.28 mg/g CE, 0.672 to 0.909 mg/g CE and 0.042 to 0.069 mg/g CE, respectively, in the hybrids grown at location of Bečej. On the other hand, female inbred originated from the BSSS population had a higher effect on the content of total carotenoids and β + γ -tocopherol in grain of hybrids. On the average, the contents were 17.80 μ g/g β CE and 27.23 μ g/g, respectively, in the hybrids at Zemun Polje and 20.43 μ g/g β CE and 19.76 μ g/g, respectively, in the hybrids at Bečej. The results indicate that there is genetic diversity in content of specific health beneficial phytochemicals among tested genotypes.

Session IV
New Tools and Technologies

4.1 Prediction of testcross performance in maize with high density SNP data

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This is a large scale study on genome-based prediction of testcross performance in an advanced cycle breeding population of maize. The analyzed data set comprised testcross progenies of 698 doubled haploid (DH) lines phenotyped for two traits, grain yield and grain dry matter content, and genotyped with 56,110 SNP markers. For the prediction of genomic testcross values, we used statistical methods differing in the modeling of kinship between DH lines and the estimation of testcross effects, e.g. best linear unbiased prediction, Bayesian Lasso and Bayes B as a variable selection method. The predictive ability of the different models was evaluated with cross-validation. Average predictive abilities were high even for a complex trait like grain yield (0.64). The results from this study are encouraging with respect to genome-based prediction of the genetic value of untested lines in advanced cycle breeding populations and the implementation of genomic selection in the breeding process.

4.2 Transcriptional profiling of maize genotypes with different drought tolerances – new perspectives for gene expression markers selection

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Drought is considered to be one of the greatest limiting factors in agriculture. Therefore, the ability of plants to adapt to drought conditions is crucial to sustain worldwide crop production. The aim of this work was to identify gene expression markers for drought resistance in maize with the potential to assist breeding. Selected maize genotypes were characterized with respect to drought resistance by measurements of the relative water content after drought stress conditions. Resistant genotypes were screened for up-regulated genes in drought conditions detected by previous microarray hybridizations. Five cDNAs coding for drought inducible genes in maize were identified and further characterized by semi-quantitative reverse transcription polymerase chain reactions. The expression levels of two of them (MZ00023411 and MZ00037881) allow a significant discrimination between resistant and susceptible genotypes. In resistant genotypes (MK01, MK01xRF7, 002x250) the expression level of these genes was at least twice higher as compared to susceptible genotypes throughout all conditions tested. Almost for all resistant genotypes were registered significant differences ($P \leq 0.05$), versus susceptible ones, in expression of these genes. Specific primers for these two genes allow discrimination of drought resistant and susceptible maize genotypes even after 2h of dehydration stress based on expression level and are suitable as potential gene expression markers, associated with drought resistance.

4.3 Physiological and molecular analysis of drought response in two sweet sorghum genotypes

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Sweet sorghum is a multipurpose crop that, providing food, feed and fuel with a limited use of resources, responds to the criteria of modern agriculture and particularly to sustainable bioenergy production. It is a C4 plant adapted to marginal soils and semi-arid environments, characteristics that should be maintained and further improved in the process of obtaining new genotypes for bioenergy production. To understand the molecular and physiological basis of drought tolerance in sorghum, two genotypes (IS 19453 and Keller) were evaluated in a growth chamber experiment. Drought stress started when plants had consumed 80% of transpirable soil water. Total RNA was extracted from irrigated and not irrigated plants at 3 levels of water stress, and gene expression analysis was carried out using microarray technique. In both genotypes the number of differentially expressed genes increased with the stress level. Most of the up regulated genes were involved in cell rescue, transport, nucleic acid binding, and in lipid, protein and sugar metabolism. In non-irrigated plants, at the higher stress level, 54 up-regulated genes presented levels of expression 2-5 fold higher with respect to control samples. Among these 54 genes, 12 were up-regulated from the onset of stress. Further experiments are being carried out to confirm gene expression analysis with q-RT PCR and to isolate candidate genes for drought tolerance.

4.4 *Agrobacterium*-mediated genetic transformation of sorghum using *in vitro* and *in planta* approaches

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In spite of great number of publications, genetic transformation of sorghum still remains a problem because of its low frequency and intensive silencing of transgenes. Using the *A.tumefaciens* strain AGL0/p35SGIB (Pniewski, Kapusta: J Appl Genet, 2005, 46: 139-147), with the *bar* and *gus-intron* genes, and two cultivars, KVV-114 and Milo-10, we studied different approaches for genetic transformation of the grain sorghum: *in planta*, by inoculation of flowering panicles (Elkonin et al., 2004: Patent RU №2229793), and widely used *in vitro* technique, by inoculation of immature embryos. In both approaches, for activation of *vir*-genes agrobacterial cell suspension was grown in the AB or CIB media with acetosyringone at room temperature. *In vitro* culture approach was effective for obtaining transgenic plants in the cv. Milo-10, which produced rapidly growing embryogenic callus from pre-cultured immature embryos after their co-cultivation with agrobacterial cell suspension and subsequent cultivation on the M11A1P2 medium (Elkonin, Pakhomova, PCTOC, 2000, 61: 115-123). Key steps for regeneration of transgenic plants were the low level of PPT during selection, omitting selective agent (PPT) from regeneration medium and addition of 6-BAP to regeneration or pre-regeneration media. Using this approach the frequency of immature embryos producing PCR-positive transgenic plants was 4.5%. Immature embryos of KVV-114 did not produce embryogenic callus, and in this cultivar transgenic plants were obtained by inoculation of flowering panicles, which was performed every day at panicle anthesis. In the progeny of each inoculated panicle the frequency of fertile PCR-positive transgenic plants survived BASTA application was approx. 1%.

4.5 Bridging the productivity gap between maize inbreds and hybrids via selection of plants excelling in crop yield genetic potential

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To overcome the yield barriers in maize, the 50 to 60% productivity gap between inbreds and hybrids should be reduced. Two selection equations $A = (x/\bar{x}_r)^2 \cdot (\bar{x}/s)^2$ and $B = (\bar{x}/\bar{x}_t)^2 \cdot (\bar{x}/s)^2$ where x is the single-plant yield, \bar{x}_r is the average yield of the surrounding plants within a moving ring of a chosen size, \bar{x}_t is the overall experimental mean, and \bar{x} and s are the progeny line mean and standard deviation. The two equations estimating with accuracy the crop yield genetic potential of single plants and entries respectively, optimize the selection efficiency by allowing application of ultra-high selection pressures (1 to 0.5%). The two-parameter selection equations, applied on plants grown at ultra-wide plant spacing, utilize the first parameter to assess the yield potential, and the second parameter to assess the coefficient of homeostasis, converting thus the yield potential into crop yield potential. In this study, the two equations were applied in an extensively cultivated in Greece maize hybrid (i.e., "Costanza") throughout the $F_2 \rightarrow F_5$ generations under open-pollination and ultra-low density (i.e., 0.74 plants/m²) conditions. Progeny testing via equation B evidenced that the productivity gap of 80% in the F_2 was reduced across two locations to 54, 20 and up to 8% in the F_3 , F_4 , and F_5 , respectively. The high selection pressures applied led to genetically homogeneous breeding lines whose top plants reached and surpassed the productivity and stability of the hybrid "Costanza". Utilization of these lines in the production of hybrids is expected to increase the crop yield potential and ensure an endless improvement of parental lines which may be constantly upgraded through nonstop selection.

4.6 Genetic diversity of maize inbred lines and grain yield of their test-crosses in stress and non-stress conditions

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The main goal of modern maize breeding is to find hybrid combinations with superior agronomic performances under broad range of growing conditions including drought and low soil fertility. Implementations of molecular markers in maize breeding programs can help in attaining this goal by providing better insight into germplasm diversity. We evaluated 16 elite maize inbred lines crossed to four testers under stress and non-stress conditions to (i) estimate the combining ability for grain yield (GY), (ii) assess the genetic diversity among inbred lines using 53 SSR markers, and (iii) estimate the correlation between F1 hybrid performance and genetic distance (GD). The F1 hybrids were evaluated under moderate drought stress, low N stress, and non stress conditions from 2007 to 2009. Mean grain yield for hybrids ranged from 3.72 Mg ha⁻¹ under moderate drought stress in 2007 to 7.73 Mg ha⁻¹ under non-stress conditions in the same year. General combining ability (GCA) effects were significant under all conditions whereas specific combining ability (SCA) effects were significant under all conditions except under moderate drought in 2007. The 53 SSR markers revealed in total 197 alleles among 20 inbred lines with 3.7 alleles per locus and a mean polymorphism information content (PIC) value of 0.55. Cluster analysis based on genetic similarity (GS) calculated using SSR marker data grouped lines mostly according to their pedigrees. Correlation coefficients between F1 GY and SSR based GD varied from 0.51 for low N stress in 2007 to 0.77 for the same stress in 2009.

4.7 Heritability and correlation of some characters in F₁ hybrids of maize in vitro

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The purpose of this study was to determine heritability and correlation of such characters as callusogenesis, somatic embryogenesis and plant regeneration. 9 inbred lines (Co125, 459, A239, 092, MK01, MK159, P502, P101, MK390) and 20 hybrids F₁ were used as experimental material. Tissue cultures were initiated from 12-13 day-old immature embryos. The data were processed by analysis of variance and coefficients of heritability for the characters under study were calculated. For the comparison of the relationships between the processes of callusogenesis, somatic embryogenesis and plant regeneration correlation coefficients were determined among parental inbred lines as well as among hybrid combinations. Highly significant differences of the frequency of embryogenic callus formation and plant regeneration process among F₁ hybrids were found out. Correlation analysis showed that for all the inbred lines (parental forms for F₁ hybrids) between the somatic embryogenesis and plant regeneration processes a positive correlation was found ($r = 0,69$ $P < 0,05$). At the same time, for F₁ hybrids the correlation between somatic embryogenesis frequency and plant regeneration frequency was much stronger ($r = 0,95$ $P < 0,001$). It was shown that male parent and interaction of both parents had the highest values of heritability for callusogenesis, somatic embryogenesis and plant regeneration processes. Female parent had the lowest heritability value and this coefficient was significant only for non-embryogenic callus formation process. These results can be used in the elaboration of new and improved methods of obtaining maize regenerants.

4.8 Resistance to fungal infection in maize is based on innate mechanisms of defense

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We investigated global gene expression in maize ears at several time points after infection with *Fusarium verticillioides*. In kernels at 48 h post infection with a fumonisin-producing strain, about 800 differentially expressed sequences were identified and nearly 10% assigned to the category cell rescue, defence and virulence. The expression analysis was extended to early (12, 24 h) and late (72, 96 h) phases after infection with a fumonisin-nonproducing strain. The mutant strain was able to activate host defence genes later than the wild type strain. When resistant and susceptible maize genotypes were compared, in the resistant lines the expression of defence genes were induced upon infection, indicative of a basal defence response against the fungus. In the susceptible genotypes defence genes were induced specifically after pathogen infection. The basal defence response was also active against several fungal species invading maize kernels.

4.9 Combining ability, genetic distance and heterosis among maize inbred lines

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The aim of this study were to investigate the relationship between genetic distance based on SSR markers with grain yield heteorsis and combining ability. A diallel cross between six maize inbred lines was carried out to estimate genetic parameters for grain yield and determine the heterosis and combining abilities of the inbreds and their crosses. Parent inbred lines and 30 F₁ crosses were analysed by RCB design in two densities (44.640 and 64.935 plants/ha⁻¹) with four replication at Zemun Polje in three years. The highest heterosis for yield was detected in the combination ZPL17 x ZPL680 while the lowest one was determined in the combination ZPL142xZPL357. Twenty nine SSR markers produced 75 alleles of which 27 are polymorphic. This gave an average 2,6 alleles per locus. Genetic distance based on Jaccard coefficient ranged from 0,52 to 0,83 (ZPL142 x ZPL17). The lowest genetic distance as well as the lowest grain yield and SCA value was established between inbred lines ZPL17 and ZPL 173. The cluster analysis based on genetic distance for SSR data clasiffies inbred lines into two principal heterotic groups. The correlation between specific combining ability and genetic distance as well as between grain yield heterosis and genetic distance was positive and significant. Althrought SSR determined genetic distances were not useful to predict hybrid grain yield heterosis they effectively grouped the lines according to heterotic pattern.

4.10 Enzymatic hydrolysis of sorghum prolamins

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Sorghum prolamins known as kafirin are characterized by their low solubility in aqueous solutions and their low digestibility. In order to improve these properties (techno-functional and bio-functional), and to extend the applicability of sorghum proteins particularly the prolamins in various food and non-food domains, enzymatic hydrolysis is carried out. For this purpose, several proteolytic enzymes with various specificities were used (alcalase from *Bacillus licheniformis*, esperase from *Bacillus lentus*, flavourzyme from *Aspergillus oryzae*, papain from *Papaya latex* and trypsin from bovine pancreas). The sorghum sample labeled AS1 from Algerian local cultivar (Tafsout el beida) was harvested in 2009 in the Algerian arid Sahara area of the South, *i.e.* Ain Salah. The kafirins were extracted with 1.0% (v/v) β -mercaptoethanol in 70% ethanol at 60°C. The purity of kafirin fraction was confirmed by SDS-PAGE. The kafirin fraction was incubated with various enzymes under pH-stat conditions [3.5 % (wprotein/v) kafirin; 5.0% enzyme (w/wprotein); pH 8.0, 50°C, 2.0 h]. The degree of hydrolysis was calculated using the pH-stat method described by Adler-Nissen. The lyophilized hydrolyzates were characterized by size exclusion HPLC, SDS-PAGE, protein contents were determined by Lowry's colorimetric method and the total nitrogen was determined by dumas combustion method using a 6.25 conversion factor. Enzymatic hydrolysis of sorghum prolamins showed that the choice of the suitable enzyme could direct the hydrolysis towards different applications. Alcalase and esperase are recommended for advanced hydrolysis for bioactive peptide purification whereas; papain, trypsin and flavourzyme are required for limited hydrolysis to improve the functional properties of kafirins.

4.11 Pseudo doubled haploid lines in maize

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During the last decade, doubled haploid technology has widely been used in maize breeding and research. This technology accelerates the production of homozygous lines (DH-lines), and it is considered that DH-lines possess complete homozygosity. However, within some DH-lines, we have noticed a significant increase of variability for main quantitative traits after each subsequent generation. Three possible reasons of this kind of phenomenon have been proposed and studied: (1) influence of the environmental conditions, (2) influence of the reagents used for chromosome doubling, (3) influence of some factors connected with haploid induction. The presented data showed that the noticed lines, called pseudo DH-lines, are not a homozygous material. The first two reasons mentioned above have appeared to be not significant for the occurrence of pseudo DH-lines. Most likely, the answer has to be found in the area of haploid induction; some confirmations of that have already been obtained in our previous studies.

4.12 Malate dehydrogenase associated to the cell walls of maize roots

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Malate dehydrogenase (MDH) (EC 1.1.1.37), regulating the synthesis and oxidation of malate is one of the key enzymes in malate metabolism. Isolated cell walls from maize roots exhibited NAD-specific MDH activity, ionically and covalently bound. The enzyme catalyses a rapid reduction of oxaloacetate and much slower oxidation of malate. Much higher activity of cell-wall associated MDH was detected in the intensively growing lateral roots compared to primary root segments with decreased growth rates. Exposure of maize plants to toxic concentrations of Zn^{2+} and Cu^{2+} in the hydroponic solution inhibited lateral root growth, decreased MDH activity and changed MDH isoforms eletrophoretic profile. The results presented indicate that cell wall MDH might be involved in the developmental processes, and possibly in detoxification of heavy metals.

4.13 Productivity levels of maize hybrids with different portion of cms

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Two maize hybrids – Kn 619 and Kn 517 were tested 2 years (2009-2010) at two plant densities including 3 portions of CMS (“mixed scheme”) – 33%, 50% and 66% of C and S type, respectively for both varieties. Regardless of xenia effect and GxE interaction, the variants with 50% CMS portion surpassed grain yield of the fertile checks – from 8 to 11% respectively for both hybrids. As an average level from all blended variants the outyielding is 3-4% for Kn 517 and Kn 619, compared even with their sterile checks (100% CMS). The experiment was enlarged with 3 other hybrids in 2010. The mean results from first year investigation are from 6,4% to 11,4% higher grain yield for the entries with 66% CMS portion compared to their fertile checks. The preliminary conclusions of that study are in a good accordance with other authors. So called “plus hybrids” system after Weingarthner et al. (2002) is very promising for farmers. The seed production of Kn 619 in 2010 was done in this way – with 66% share of CMS.

4.14 Combining ability of *in vitro* doubled haploid maize lines

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In the case of maize breeding the use of microspore-derived tissue culture makes it possible to produce lines that are almost 100% homozygous within a year. The application of this method allows breeders to respond promptly to changes in market demands. An efficient *in vitro* tissue (anther) culture system has been developed for maize in our institute, allowing a large number of doubled haploid (DH) lines to be developed each year. The aim of the present work was to analyse the combining ability of the DH maize lines developed using this system in order to clarify the role these lines could play in field maize production. Field performances of maize hybrids with DH background were examined in Martonvásár. The performance of thirty-six hybrid combinations was compared with that of two standard maize hybrids with commercial value. Compared with the grand mean of the experiment, the majority of hybrids performed well below the level of the standard mean, though it was possible to find a DH line that resulted in hybrids capable of producing yields equivalent to that of the standards. The importance of these results is that the DH lines were developed within an *in vitro* plant regeneration system and were tested in performance trials after long years of selection. Up till now only DH lines developed *in vivo* have been introduced into cultivation. If the studies are extended to include a wider range of genetic sources, the *in vitro* DH technique could be utilised for the purposes of hybrid development.

4.15 Efficiency of chromosomes doubling in maize maternal haploids

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The results represent a summary of the three year study (2008-2010) on the efficiency of chromosomes doubling procedure in maize maternal haploids. The maternal haploids were derived from 22 different maternal genotypes. Induction of maternal haploid was performed with RWS9 inducer, obtained from Prof. H. H. Geiger (University of Hohenheim). During three years study, the most effective in chromosomes doubling variants were: colchicine concentration 0,08% and the exposure times 8 and 10h. Considerable differences were noticed in haploids doubling depending on their maternal genotypes.

Session V

Resource Allocation, Educational and Human Resources, Miscellaneous

5.1 Resource allocation in a maize breeding program for native resistance to western corn rootworm

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Maize breeding programs for native resistance to western corn rootworm (WCR), *Diabrotica virgifera virgifera* are resource intensive due to labor-consuming digging up the maize root and large scale field-based germplasm screening. Therefore, it is mandatory that the available resources are spent in an optimum way. At the Agricultural institute Osijek, Croatia, 127 maize genotypes were screened in 2007, 2008, 2009 for root damage caused by WCR larvae, root size and superior secondary root growth after the damage. The experiment was conducted in two replications as an alpha design planted at two locations under continuous maize growing conditions in Eastern Croatia, a major maize production area with natural WCR occurrence. In addition to sources of genetic variability, we used estimates of heritability and information on the relative magnitudes of genetic, genetic x environmental, and error variances to optimize allocation of resources in the field-based WCR native resistance breeding programs. Adjusted means from individual experiments were combined in an across-environments analysis to obtain estimates of the year variance, location variance, genotypic variance, corresponding interaction variances and the residual variance. The residual variance included between-plot error variance, within-plot environmental variance and within-plot genetic variance with n=number of plants (roots) sampled per plot. How these parameters impact heritability for three WCR-related traits and the efficiency of breeding program to improve WCR native resistance will be discussed.

5.2 Performance of five maize inbred lines in crosses to three Lancaster testers

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Five inbred lines of BSSS background were crossed to three testers of Lancaster background in order to evaluate their performance through developed hybrids. The two-replicate trial was set on three locations according to the randomised complete-block design (RCBD) in 2010. The following traits were observed: grain yield and yield components (ear length, number of kernel rows, number of kernels per row, kernel depth and 1000-kernel weight). ANOVA for RCBD was done for all obtained data, while individual comparisons were performed by the LSD test. Hierarchical cluster analysis of traits of both, inbreds and hybrids, was done and dendrograms for classification of genotypes of similar performances were made. ANOVA showed a significant effect of a genotype and a location at the level of $P=0.01$. LSD test showed significance of differences among certain genotypes. Grain yields significantly varied at level of $P=0.05$. Within inbreds the highest grain yield gave inbred A1, and the lowest inbred Z2, while within hybrids A4xZ4 gave the highest, and A5xZ2 gave the lowest grain yield. According to LSD test, the combinations of inbreds with the tester Z3 showed significantly better performances than other testers with same inbreds. This tester *per se* was better than other testers and was statistically significant for the observed traits. Hybrids with a tester Z3 as a male component were grouped by the cluster analysis into one subcluster in dendrograms. This tester was separated into a specific subcluster in the dendrogram obtained by the cluster analysis of the inbreds *per se*.

5.3 The aspect of biological control of *Beauveria bassiana* activities in regulating a population level of European corn borer (*Ostrinia nubilalis*)

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A favourable ecotoxicological aspect of the fungus *B. bassiana* (Balsamo) Vuillemin and a high degree of virulence in insects are the optimum solutions and one of the most desirable approaches with a view to reduce abundance of pests. The aspect of biological control was observed through effects of different treatments in dependence on the maize developmental stage: (1) seed treatment with the granular formulation of conidia and with the insecticide Cruiser applied immediately before the sowing, (2) foliar application and injection into the stalk of the fungal suspension in the concentration of 2×10^8 conidia per ml in the 7-leaf whorl-stage (V 7) and (3) application of the granular formation of spores on the ear in the silking stage (R1). The fungal efficiency in regulation of the population level of European corn borer (*Ostrinia nubilalis*) was tested under field and laboratory conditions during 2009. All plants were analysed at harvest and it was determined that plants foliarly treated were the most damaged by the ECB larvae (38.18% under natural attack and 66% under artificial inoculation). As expected, the insecticide Cruiser was the most efficient with the attack percentage of 33.80% and 42.87%, while this percentage amounted to 36.63% and 55.55% under natural attack and artificial inoculation with ECB egg broods, respectively. Under laboratory conditions, *O. nubilalis* larvae were treated with fungal mycelia and spores in the powder formulation, whereby larval mortality was 100% when the isolates D-21 and BB-3 were applied after seven and 10 days, respectively.

5.4 Optimum allocation of resources in joint linkage-association mapping studies determined with computer simulations

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Joint linkage-association mapping (JLAM) approaches have been suggested as a promising strategy to map QTL in plant populations. The objective of our study was to use intensive computer simulations to investigate the optimal allocation of resources (number of parents, mating design, number of families, family size, and number of environments used for phenotyping) for QTL detection in JLAM based on diallel mating (DAM) and single round robin (SRR) designs. Our results suggest that the power to detect QTL can be substantially increased by optimal allocation of resources in JLAM. Optimal allocation represents a compromise between high heritability on an entry mean basis and a large size of the mapping population based on several parents to guarantee high probability of segregation of QTL.

5.5 Combining abilities of the new Bc maize inbred lines

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Seven new maize inbred lines were selected from the Bc Institute maize breeding program through tests with the line tester Mo17 (Bc11-7, Bc63473-13, Bc65073-4, Bc65741-28, Bc69110, Bc69111 and Bc720964). Combining ability of these lines was estimated from hybrid combinations with three testers (Bc1511, Bc84 and Pa91) tested in field trial in Rugvica in 2010. Lines Bc11-7, Bc63473-13 and Bc65073-4 along with testers Bc1511 and Pa91 had positive effects for grain yield. The hybrid combination Bc720964 x Bc1511 had the highest grain yield 12,39 t/ha with the SCA effect of 0,715. Negative effect for grain yield was observed for tester B84, parent of low yielding hybrids except for the combination with line Bc11-7 whose SCA was highest (1,177). This indicates that tester B84 was related to all investigated lines, excluding Bc11-7. Economically, the most valuable hybrid combinations were crosses with tester Bc 1511, one with the highest GCA effect (1,140) for grain yield. Furthermore, an interesting combination is Bc11-7 x Bc1511 with the lowest grain moisture (27,30 %) and the grain yield 11,61 t/ha. However, the true value of the investigated combinations should be checked at more appropriate environment for FAO 600.

5.6 Improvement of data from highly heterogeneous sorghum field trials in mali employing geo-statistical methods

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A growing world population juxtaposed with diminishing fertilizer resources e.g. phosphorous presents new challenges for current and future plant breeding. The burden of low-input conditions is particularly felt in Sub-Saharan Africa as many farmers don't have access to fertilizer. Plant breeding for adaptation to low-input conditions presents an opportunity to increase yields on farm level. Spatial variability of plant nutrients and other growth factors is mostly higher in low-input field trials. A four year multi-location experiment was conducted in Mali, West Africa. Seventy sorghum genotypes were evaluated under low and high input conditions. High spatial heterogeneity within each trial was observed for plant available soil P content and different plant traits. Therefore spatial adjustment models were applied. Data for grain yield were analyzed in each environment with 92 different models including 82 models with autoregressive spatial correlation terms. Spatial models (AR1, AR2) could significantly improve broad sense heritability and relative efficiency of grain yield compared to randomized complete block (RCB) and lattice designs. No specific model was best for all environments. Spatial modeling lead to different genotype ranking for grain yield compared to RCB. Improvement of heritability and efficiency was greater in low-input trials and trials with few replications. Thus the use of spatial models is recommendable for trials with high soil heterogeneity and low broad sense heritability values and therefore an important tool especially for plant breeding trials under low-input conditions and trials with few replications.

5.7 Evaluation of NS maize hybrids in multilocation trials using AMMI and GGE biplot analysis

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Multienvironment yield trials are essential in estimation of genotype by environment interaction and identification of superior genotypes in the final selection cycles. The objective of this study was to evaluate stability and adaptability of grain yield of experimental maize hybrids by GGE (Genotype and Genotype by Environment Interaction) biplot and AMMI (Additive Main Effects and Multiplicative Interaction) analyses. This study comprised 20 experimental NS maize hybrids tested across five locations in northern Serbia in a randomized complete block design. The ANOVA indicated significant effects of genotypes, environments and their interaction, their proportion in total yield variation being 12,9%, 70,3%, 16,8% respectively. Joint effects of genotype and interaction (G+GE) was partitioned using GGE biplot analyses where the first two components were significant, explaining 70,9% (45,8% PC1 and 25,2% PC2) of GGE sum of squares. The first two principal components in AMMI analysis were significant, explaining 78,58% (45,45% PC1 and 33,13% PC2) of interaction variation. Both analysis distinguished the same four genotypes with differences in ranks. Stability of analysed genotypes was similar in both methods, with few exceptions (i.e. G6 and G20). It is concluded that there is no difference between the AMMI and GGE biplot analysis in evaluation of experimental maize hybrids in different climatic conditions and that both methods can be used equally successful.

5.8 Effect of manure supplemented with phosphate fertilizer on the fodder yield and quality of two sorghum cultivars (*Sorghum bicolor* L.)

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Two sorghum cultivars (Izaa-7 and Razini) were subjected to manure (0, 10, 20 or 30 t ha⁻¹) and phosphate fertilizer 0, 28 or 56 kg P₂O₅ha⁻¹) in northeastern part of Jordan. A progressive increase in fodder and Stover yield was observed with manure and P application up to higher rates. This increase in fodder (dry matter) yield observed was related to the greater plant height, stem diameter and leaf area development in response to manure and P application. Quality parameters such as ash percent and P contents were increased due to manure application whereas, protein content showed variable response to manure and P application. Overall, higher fodder yield and quality was recorded in cv Izaa-7. For the two cultivars, 20 t manure and 56 kg ha⁻¹ seems to be the best rates for harvesting higher fodder yield of sorghum with good quality under Jordan conditions.

5.9 *Fusarium* ear rot and mycotoxin content in genetically modified maize grown in Poland

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The aim of this studies was comparison of incidence and severity of *Fusarium* ear rot in both Bt and non-Bt near-isogenic maize varieties and comparison of mycotoxins concentration in kernels of transgenic (Bt) and non-transgenic maize grown is different locations in Poland. Experiments were conducted in years 2008-2010 in South-Western and Eastern Poland where European cornborer is a serious problem. In each experiment three 1-hectare plots were sown. Hybrid variety DKC 3420 (chemically protected against *O. nubilalis*, and control without protection) and isogenic Bt variety DKC 3421 were sown. Cobs- and kernel samples were collected from the field experiments in Autumn. Cobs were evaluated for *Fusarium* ear rot severity using 0-5 rating scale. Representative samples of grain were grinded in laboratory mill and mycotoxins: deoxynivalenol (DON) and fumonisins (FB₁, FB₂, FB₃) were determined after extraction with proper solvent (acetonitrile:water (84:16) or methanol) and purification on SPE columns. Analyses were conducted on HPLC system equipped with UV-VIS (DON) and fluorescence detector (fumonisins) and RP C18 HPLC column. The content of ergosterol as a marker of presence of mycelia was analyzed on HPLC after microwave-assisted hydrolysis and extraction with n-pentane. Evaluation of ear rot symptoms and yield of grain showed similar results in both locations. Yield of grain of DKC 3421 YG and chemically protected DKC 3420 was higher than in not protected DKC 3420 (control), but only in location A the difference was statistically significant. Mean grain yield in location A was significantly lower (72,3 g/cob) than in location B (112,9 g/cob). *Fusarium* ear rot severity was also significantly higher in control field without protection compared to chemically protected or Bt variety. Mycotoxin analysis revealed presence of deoxynivalenol and lack of fumonisins. DON concentration didn't exceeded limit (1,75 mg/kg grain) in DKC 3421 YG and in chemically protected DKC 3420. The mycotoxin content was exceeded only in control sample from location B in Eastern Poland. Ergosterol content supported data from mycotoxin analysis, especially at higher DON concentrations.

5.10 Assessment of grain maize fields and certified seed production in different categories in Iran

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These days, one of the most important ways to achieve sustainable production of maize in the world and especially in Iran is usage of certified and healthy seeds. In the current decades, in order to prevent mixing of certified seeds with contaminated seeds and also achieve to healthy seed, control and certification systems are invented and performed in developed countries. In this regard, the main purposes of seed quality controlling are preventing of entry of exotic pests and diseases and also reducing of spreading of them. On the other hand, the goal of evaluation of grain maize farms is determining the categories and different classes based on minimum percentage of seed infection to diseases, pests and genetic purity in each seed categories or classes. In control and certification system of maize, seed categories are including: seed primary nucleus, maternal category and certificated seeds which each of them are divided into different seed classes. Maize breeder selection, farm visiting, primary and finally visitation, disease diagnosis, pest and weed assessment, sampling, emasculation or remove of tassel, seed tests and certificate label installation are different stages of controlling and certifying of maize seed in Iran.

5.11 Functional and molecular characterization of ZmSnRK2.8 and its interactors

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Plants grow in a dynamic environment that frequently imposes constraints on growth, development and productivity. Abscisic acid (ABA) plays a major role in regulating several developmental and physiological processes, such as seed maturation and germination, and mediating the responses of vegetative tissues to osmotic stress. ABA levels increase in late embryo development shortly before the onset of desiccation and in vegetative tissues under water-deficit stress conditions. ABA triggers both stomata closure and water-deficit tolerance responses by regulating changes in the activity of ion channels and changes in gene expression. Protein kinases are key components for mediating cellular responses, including responses to osmotic changes. Many kinases have been described to be involved in ABA and/or osmotic signalling affecting stomata function and/or gene expression. We have isolated a maize SnRK2 kinase that is constitutive, rapidly activated by ABA and osmotic stress in vegetative tissues and that is capable of complementing the *Arabidopsis ost1-2* mutant, which is defective in the regulation of transpiration upon water stress. Using a yeast two hybrid system we have identified two new types of transcription factors that are potential substrates for this enzyme (ZmbHLH and ZmSNAC1). Transcription factors are interesting study candidates to unravel the molecular mechanisms of SnRK2 kinases since they control cellular adaptation by regulating gene expression and represent important tools for biotechnological crop improvement. Understanding these complex pathways of ABA signalling and pinpointing important proteins of drought response could prove to be fundamental for future breeding approaches applied to face global climate change.

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