Eucarpia 2013

30th EUCARPIA Fodder Crops and Amenity Grasses Section Meeting

Quantitative traits breeding for multifunctional grasslands and turf

Book of Abstracts

12th to 16th May 2013
Congress Center - Hotel Zvezda
Vrnjačka Banja - SERBIA

INSTITUTE FOR FORAGE CROPS
KRUŠEVAC

Eucarpia 2013

30th EUCARPIA fodder crops and amenity grasses section meeting “Quantitative traits breeding for multifunctional grasslands and turf”

12th to 16th May 2013 Vrnjačka Banja, Serbia

30th Meeting of the EUCARPIA Fodder Crops and Amenity Grasses Section

“Quantitative traits breeding for multifunctional grasslands and turf”

BOOK OF ABSTRACTS

Vrnjačka Banja, Serbia

12th - 16th May 2013

Institute for forage crops, Kruševac

37251 Globoder, Serbia
Research and development Institute

General scientific area
Biotechnology and Agriculture Science

Research fields

- Genetics, physiology and breeding of the perennial legumes and grasses;
- Collecting, studying and preserving of the forage crops genetic resources;
- Production and utilization of the forage crops from arable lands and grasslands;
- Conservation of herbage and research on biomass quality;
- Improvement of the forage crop seed production and pollination with honey bee.

Services

- Knowledge transfer from research areas of animal feed production, conserving and utilization to farmers;
- Analyses of chemical composition of herbage and concentrate animal feed;
- Analyses of soil chemical composition.

Commercial activities

- Production, processing and trading of the seed of perennial legumes and grasses;
- Production of the bred queen bees, honey and candy for bee feeding;

This meeting has been organized with support of

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INSTITUTE FOR FORAGE CROPS
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Institute for forage crops,

37251, Globoder, Kruševac, Serbia

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Introduction

Plant breeding is applied science. Science is the pursuit of knowledge. In order to advance, plant breeding needs new knowledge and the creative, innovative application of existing knowledge within existing or new frameworks. The actors are both storming young scientists as well as experienced breeders.
Young scientists usually are keen to transgress borders. Plenty of experienced breeders have been at the other side of the border for a while but bounced back in due time, loaded with matured ideas and concepts.

A balanced combination of the boldness of the dreaming youngsters and the prudence and realism of the experienced ones, usually holds the best opportunities to build successful research and breeding programmes.

With the support of the scientific committee the organizers tried to reflect that equilibrium and succeeded in composing an attractive programme, mixing the building blocks of plant breeding research centered around the challenging topic of the Meeting: “Quantitative traits breeding for multifunctional grasslands and turf”.

The 30th Meeting of the *EUCARPIA* Fodder Crops and Amenity Grasses Section offers an unique forum to present, share and assess diverging ideas, breakthroughs and progresses in this field of interest.

This book of abstract presents the key findings. Up to the delegates to deepen and widen the perspectives and to take home whatever may fit into their activities. Of course with the idea to look back within a number of years to find out how much of it has been realized and how much of it really has provoked or produced a benefit for the world.

In the name of all participants I deeply express my feelings of gratitude and appreciation to the Serbian colleagues of the Institute for forage crops, Kruševac to take the initiative and the lead in the organization of the Section Meeting.

Those among us who organized previous meetings know how much energy and dedication the organization demands. It is a journey full of foreseeable and unforeseeable obstacles but at the end there is a priceless reward: the certitude that the meetings of our Section offer to all participants a variety of aspects that leaves no mind unchanged.

May, 2013

Dirk Reheul,

Chairman of the EUCARPIA Section “Fodder Crops and Amenity Grasses”

Contents

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Programme of 30th Meeting of EUCARPIA Fodder Crops and Amenity Grasses Section

Sunday, 12 May

14:30-17:30 ***Festulolium Workshop / 3rd Festulolium Working Group Meeting*** (*Small hall*)

18:00-20:00 Registration and poster setting
20:00 Welcome reception

Monday, 13 May

08:00-09:00 ***Registration and poster setting***

09:00-09:45 ***Opening ceremony*** - Welcome addresses (*Main conference auditorium*)

Representative of Ministry of education, science and technological development
Representative of Ministry of Agriculture, forestry and water management
Dirk Reheul - Chairman of EUCARPIA Fodder Crops and Amenity Grasses Section
Beat Boller – Chairman of EUCARPIA
Zoran Lugić – Manager of Institute for forage crops, Kruševac

09:45-11:00 ***Introductive lectures*** (*Main conference auditorium*)
Chairpersons: Dirk Reheul, University of Gent, Belgium and Zoran Lugić, Institute for forage crops, Kruševac, Serbia

09:45-10:15 Introductive lecture: *Grasslands and forage crops in Serbia: production and breeding.* Dejan Sokolović, Jasmina Radović, Zoran Lugić (Institute for forage crops, Krusevac, Serbia), Aleksandar Simić (Agriculture faculty, Belgrade University, Serbia)

10:15-11:00 Introductive lecture: *Forage and grasslands in a sustainable agriculture. New challenges for breeding.* Christian Huyghe (INRA, France) and Charles Brummer (Noble Foundation, USA)

11:00-11:40 Opening Cocktail

11:40-19:20 ***Session1 “Genetic variability in forage productivity and quality and its exploitation through breeding”*** (*Main conference auditorium*)

11:40-13:10 ***Invited papers Session 1*** *“The value of genetic resources for the improvement of complex traits”*
Chairperson: Luciano Pecetti, CRA-FLC, Lodi, Italy

11:40-12:25 Invited paper: *Ex situ conservation of genetic resources of forage and turf species.* Evelin Willner (IPK Genbank, Malchow, Germany)

12:25-13:10 Invited paper: Characterization of alfalfa *Medicago sativa* genetic resources. Isaak Rashal and Dace Grauda (Institute of Biology, University of Latvia, Latvia)

13:10-14:25 Lunch (*Hotel restaurant*)

14:25-15:55 ***Invited papers Session 1 bis*** *“Exploting genetic diversity for complex traits”*
(*Main conference auditorium*)
Chairperson: Daniel Thorogood, IBERS, Aberystwyth, UK

14:25-15:10 Invited paper: *Characterization of genetic diversity for resistance and quality traits using molecular tools.* Tom Ruttink, Hilde Muylle (ILVO, Belgium), Susanne Barth (Teagasc, Ireland)

15:10-15:55 Invited paper: *Association mapping approaches for characterizing complex traits in forage species*. Torben Asp (University Aarhus, Denmark)

15:55-19:20 ***Offered papers Session 1*** (*Main conference auditorium*)
Chairperson: Baldomero Alarcon-Zuniga, UAC, Texcoco, Mexico

15:55-16:15 Valentina Nanni, Marilena Paolini, Angelo Innocenti, Paolo Mulè, Mirella Vargiu (Italy): A breeding program started from contrasting genotypes of alfalfa (*Medicago sativa* L.), as characterized in the frame of the PERMED project

16:15-16:35 Mirjana Petrović, Zoran Lugić, Zora Dajić-Stevanović, Bojan Anđelković, Jordan Marković, Vladimir Zornić (Serbia): Variability of alsike clover (*Trifolium hybridum* L.) natural populations from Serbia

16:35-18:00 Coffee break with ***Poster Session 1*** *(Poster hall)*

16:45-18:00 EUCARPIA Section Board meeting (*Small hall*)

15:55-19:20 ***Offered papers Session 1*** (*Main conference auditorium*) continuation
Chairperson: Baldomero Alarcon-Zuniga, UAC, Texcoco, Mexico

18:00-18:20 Marc Ghesquière, Philippe Barre, Jean-Louis Durand, Isabelle Litrico, Jean-Paul Sampoux, Pierre Mournet, Florence Volaire (France):Genetic response to climate scenarios within *Dactylis* and *Festuca* of temperate vs Mediterranean origin.

18:20-18:40 Tomáš Vymyslický, Zdeněk Musil (Czech Republic): Monitoring of vegetation changes in selected sinkholes in the Moravian karst.

18:40-19:00 Tim Vleugels, Joost Baert, Erik van Bockstaele (Belgium): Evaluation of a diverse collection of red clover germplasm for susceptibility to clover rot (*Sclerotinia trifoliorum*) and other important traits

19:00-19:20 Poster talks Session 1

Jana Repková et al. (Czech Republic): Interspecific hybrids *Trifolium pratense* x *Trifolium medium* as the source of new diversity.
Mathias Cougnon et al. (Belgium): Factors influencing preference of tall fescue genotypes for grazing sheep.
Sanja Vasiljević et al. (Serbia): Genetic diversity of red clover (*Trifolium pratense* L.) determined by phenotypic traits and SSR molecular markers.
Daniela Knotová et al. (Czech Republic): The utilisation of wild *Fabaceae* species in grasslands.

19:30 Welcome Cocktail – ***"Serbian evening"*** *(traditional food and wine degustation)*

Tuesday, 14 May

Mid-conference tour

09:00- 09:15 Meeting and departure (infront of the hotel)

10:00-13:00 Experimental field tour in the Institute for forage crops, Kruševac

13:30-15:00 Lunch

16:15-18:15 Visit to dairy farm and Lazar - Blace

19:15-21:15 Guided tour in old town Krusevac and Cocktail in Town hall - ***Mosaic evening***

22:00 Arrival time to V. Banja. (approximately)

Wednesday, 15 May

09:00-13:30 ***Session 2 "New biotechnology methods in sustainable breeding: strategies and implementation"***
"Paving the way to more efficient breeding strategies" (*Main conference auditorium*)

09:00-11:15 ***Invited papers Session 2***
Chairperson: Isabel Roldan-Ruiz, ILVO, Belgium

09:00-09:45 Invited paper: *Genomics of leaf lifespan: modifying the leaf senescence*. Karin Krupinska (Christian Albrecht University, Kiel, Germany)

09:45-10:30 Invited paper: *Functional genomics of grass reproductive traits– paving the way towards hybrid breeding in forage and turf grasses.* Bruno Studer (ETH Zurich, Switzerland)

10:30-11:15 Invited paper: *Emerging knowledge from genome sequencing of model and crop species.* Aleksandar Mikić (IFVCNS, Novi Sad, Serbia), Bernadette Julier, Frédéric Debellé (INRA, France), Luigi Cattivelli (GRC, Italy), Ian Armstead (Aberystwyth University, UK)

11:15-12:00 Coffee break with ***Poster Session 2*** *(Poster hall)*

12:00-13:30 ***Offered papers Session 2*** (*Main conference auditorium*)
Chairperson: Bernadette Julier, INRA, Lusignan, France

12:00-12:20 Dawid Perlikowski, Arkadiusz Kosmala, Izabela Pawłowicz, Marcin Rapacz, Janusz Kościelniak, Zbigniew Zwierzykowski (Poland): Influence of short-term drought conditions and subsequent re-watering on the physiology and proteome of *Lolium multiflorum* / *Festuca arundinacea* introgression forms with contrasting levels of tolerance to long-term drought

12:20-12:40 Daniel Thorogood, Matthew Hegarty, Ros Mathews, Debra Allen, James MacDuff (UK): A genetic association between leaf elongation rate and flowering time in perennial ryegrass.

12:40-13:00 Janaki Velmurugan, Dan Milbourne, Chris Creevey, Helena Meally, Linda Cardle, David Marshall, Micha Bayer, Matthew McCabe, Bridget Lynch, Susanne Barth (Ireland): A cost effective approach to generate a large number of markers for genotyping in perennial ryegrass suitable for whole genome association studies.

13:00-13:30 Poster talks Session 2

Meraluna Canunayon et al. (UK): Identification of QTLs for leaf senescence in perennial ryegrass (*Lolium perenne L*.).
Dragan Milić et al. (Serbia): Heterosis in alfalfa breeding.
Schubiger Franz Xaver et al. (Switzerland): Inheritance of crown rust resistance in a perennial ryegrass genotype of the cultivar Arvella.
Joost Baert et al. (Belgium): Variation of cell wall digestibility in fodder grasses and a perennial ryegrass breeding pool.
Kovi Mallikarjuna Rao et al., (Norway): Transcriptome profiling under hardening/de-hardening conditions in Lolium perenne by RNA-sequencing
Ottilia Nhengiwa (Germany): Breeding of rye grass varieties adapted to biotic and abiotic stress by integration of innovative genomic and metabolomic tools.

13:30-14:40 Lunch (*Hotel restaurant*)

14:40-16:10 ***Session 3 "Turf grass breeding" (invited session)*** (*Main conference auditorium*)
Chairperson: Niels Peter Roulund, DLF -TRIFOLIUM, Denmark

14:40-15:25 Invited paper: *Recent achievements in breeding for turf quality under various biotic and abiotic stress.* Trygve Aamlid (Bioforsk, Norway), Vincent Gensollen (Geves, France)

15:25-16:10 Invited paper: *Selection of grasses for lawn tennis use (interaction between variety and turf management).* Andy Newell (STRI, UK)

16:10-16:50 Coffee break

16:20-17:50 ***Workshop:*** *Public-Private Partnership - constrains and opportunities.* Moderators: Ulrich Posselt (University of Hohenheim, Germany) and Charles Brummer (Noble Foundation, USA) (*Small hall*)

17:50-18:50 ***General assembly of EUCARPIA section*** (*Main conference auditorium*)

19:45 ***Serbian traditional night followed with conference dinner***

19:45-20:30 Concert of Serbian folk dances, music and songs (Cultural association 14th October, Kruševac) (*Main conference auditorium*)

20:30 **Conference dinner** (*Hotel crystal hall*)

Thursday, 16 May

09:00-12:20 ***Session 4 “Breeding for new roles of multifunctional forage species”***
(*Main conference auditorium*)

09:00-10:30 ***Invited papers Session 4***
Chairperson: Jasmina Radović, Institute for forage crops, Kruševac, Serbia

09:00-09:45 Invited paper: *Breeding for healthy hay: Optimizing plant polyphenols in Legumes for ruminant nutrition, animal health and environmental sustainability*. Irene Muller-Harvey (University of Reading, UK)

09:45-10:30 Invited paper: *Can genome scans be combined to environmental information to improve the use of collection of natural diversity as genetic resource for forage and turf breeding.* Jean-Paul Sampoux (Inra, Lusignan, France)

10:30-10:50 ***Section history, summary of the last 10 meetings*** Ulrich Posselt (University of Hohenheim, Germany)

10:50-12:20 ***Offered papers Session 4***
Chairperson: Tomáš Vymyslický, Agricultural research, Ltd., Troubsko, Czech Republic

10:50-11:20 Joint presentation, Ulf Feuerstein, Anita Swieter (Germany): Fair evaluation of yield and quality potential in forage species evaluation; Monitoring, analysis and modeling of yield and quality dynamics of *Lolium perenne* varieties for biogas production

11:20-11:50 Coffee break with ***Poster Session 4*** *(Poster hall)*

11:50-12:10 Goran Jevtić, Bojan Anđelković, Jasmina Radović (Serbia): Effect of alfalfa cultivar on pollinator visitation, seed yield and yield components

12:10-12:20 Poster talks Session 4

Aina Ramsay et al. (UK): Condensed tannin analysis by thiolytic degradation in sainfoin (*Onobrychis viciifolia*) silage.
Dušica Delić et al. (Serbia): Effect of different rhizobacteria on alfalfa, *Medicago sativa* L. yield by inoculation of a preceding oats, *Avena sativa* L.

12:20-13:10 ***General conclusions and Closing ceremony*** *(Main conference auditorium*)

13:10-14:30 Lunch (*Hotel restaurant*)

15:00 Charter busses departure to Belgrade (*Infront of the hotel*)

15:00 Post-conference tour departure (*Infront of the hotel*)

Agriculture, forage crops and grasslands in Serbia: production and breeding

***Dejan Soković*[1], *Jasmina Radović*[1], *Zoran Lugić*[1], *Aleksandar Simić*[2]**

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Abstract

Nowadays, Serbian agriculture is the basis for economy and engine for development of rural areas. It is also the only economy sector in Serbia with a positive foreign trade balance, which means that it is the backbone of foreign trade development of country (21% share in total exports). Therefore, with considerable nature resources (agricultural land, climate, water) and capacities (farmers, livestock fund, processing structures), agriculture in Serbia has enormous potential. In Serbia farmers, representing 17.3% of the total population (more than 1.240.000 individuals), live on 631.122 farms with average size of 4.5ha. In Serbia agricultural land covers around 5.1 million ha (83% of that is cultivable).

Animal production is the most important branch of agriculture in the country, with 42% share in the total value of agricultural production. Cattle products (meat and milk) participate in value of all animal husbandry products with 42.6%. Unfortunately, number of heads in herbivore herds in the last decade has been decreasing continuously, approximately 2 to 3% annually. At the moment Serbia has 909 thousand cattle, 1729 thousand sheep and 236 thousand goats. Total meat production in Serbia has a tendency of stagnation or slight decline. Production of milk has been decreasing and in 2011 amounted to 1.434 million litres of cow milk and 11 million litres of sheep milk. Carriers of this production are family households which provide 92% of total production. Reasons for negative trend in milk and meat production are reduced local market and previous markets abroad, transition and privatisation, unstable prices and subsidies policy. Main reason for insufficient export is absence of the 3 key factors (quantity, quality and continuity in production).

Approximately 2/3 of total Serbian country is situated in the hilly-mountainous area. Especially in these areas natural meadows cover large acreage, over 1.45 million hectares, or almost 29% of total agriculture land in Serbia, while fields under the sown grasslands are on 150.000ha. In extensive forage production (dominating in Serbia), used genotypes and cultivars make difference. They should be flexible and adaptable, tolerant to drought and in the same time productive and high-quality. Developing of breeding germplasm beforehand adapted to local agro-ecological conditions (which means collecting of autochthonous populations and ecotypes from nature) is first and regularly practiced task in forage breeding in Serbia. Secondly, involvement of some specific breeding objectives, like root characteristics, filed persistency, low pH tolerance, symbiotic N fixation and tolerance to pathogens, can improve plasticity and overcome environment limitations. Both approaches will bring part of desired characteristics to new genotypes, improve yield and meet farmer needs and expectations. Recent years in two main scientific and breeding Institutes in Serbia very broad forage crops genotypes assortment is formed, well distributed among cultures and species.

What could be our future steps in order to improve fodder crops production and animal husbandry in Serbia, overall? Regionalization in forage production driven by state (recommendations for species, mixtures and cultivars which should be cropped), stabilisation of subsidy policy (introduction subsidies for certify seed production), consolidation of fields and farms especially in depopulated regions, improvement of production technologies and management on grasslands and involvement of grazing in order to make production sustainable and profitable.

Forage and grasslands in a sustainable agriculture: new challenges for breeding

C. Huyghe[1] and E.C. Brummer[2]

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Abstract

Grasslands and forage crops are a major component of cropping systems and agri-food systems worldwide. Grasslands and forage crops and the associated animal production systems play a role in a wide range of ecosystem functions, from production to environmental as well as cultural services. Their sustainability is continuously under question and it is essential to consider breeding of new varieties as a component to achieve sustainability. Several key challenges in the preservation or improvement of sustainability will be discussed. Chief among them is the need for increasing production to meet the demand of an growing population with a higher consumption of animal products, and reducing losses during the production process is particularly relevant for forage crops and grasslands. In addition, the number of farmers is rapidly decreasing and their average age is increasing. This group is generally risk averse, affecting their willingness to adopt new sustainable production systems promoted by agricultural scientists. Environmental issues to be discussed are distributed along a time scale. Short term (reducing the impact on environment), medium term (shortage of resource) and long term (climate change) issues must be considered together.

Innovation in production systems may be difficult because of lock-in situations (Geels, 2011), where a dominant socio-technical system prevails and all stakeholders are in equilibrium with this system. Innovations may then become difficult to introduce and any technological input or change in regulation may be questioned as to whether it reinforces this equilibrium or modifies it and facilitates the transition. New grassland systems or new varieties may be revisited through this grid. But it is also essential to see how to stimulate innovative conception (Hatchuel and Weil, 2012), where a continuous dialogue between concept and resource of knowledge makes it possible to envisage rupture innovations.In grasslands production, any new individual practice may be assessed for its impact on the various performances of a farm, which are indicators of sustainability. The choice of a variety (which is related to registration and breeding criteria) has an influence on a farm, but above all it is a component of a system. The question is then to identify the most adapted systems to improve overall performance and then to select the most adequate varieties in those systems. Two main points are discussed.

First, increasing the amount of forage legumes in the grassland and forage crops acreage and in ruminant diets appears essential to curtail the use of fertilizer N. It may be achieved either in the animal farms or in neighbouring cereal farms by sowing pure forage legumes (alfalfa) in rotation with cereals or by sowing grass – legume mixtures for hay or pasture.

Second, the reduction of losses during forage conservation and animal consumption is the second main component of sustainability. Although many efforts have been devoted to increasing digestibility through breeding or biotechnology, which indirectly reduces losses, little attention has been paid to the conservation issue. Identification of variety traits to reduce losses (palatability, intake during grazing, conservation) and incorporation of them into systems using advanced management strategies will be a critical issue for the future.

Finally, we will show that implementation of innovation requires their acceptability by farmers. The possibilities to improve acceptability will be discussed in general terms and we will show how relevant they are to marketing of new varieties and production systems. Currently, convincing farmers to adopt new cultivars is very difficult simply using scientific data. We will argue that on-farm demonstrations are critically important to spur adoption of new cultivars.

Ex situ conservation of genetic resources of forage and turf species in Germany

Evelin Willner and Klaus J. Dehmer

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Abstract

The IPK Genebank, Satellite Collections North, is responsible for the *ex situ* maintenance of genetic resources of forage plants in Germany. More than 10.400 accessions of grasses (forage and turf species) and 1.300 small grained forage legumes (red clover and alfalfa) belong to the collections, originating mainly from Europe, but partially also from destinations around the world. The main goal is to hold all accessions available for distribution and characterize/evaluate them for different research and breeding purposes as intensively as possible. For this means, a good documentation system is necessary, besides the management of plant genetic resources according to European Genebank Standards (Boller et al., 2005; Rao et al., 2006) and a protocol for a quality management system (certificate ISO 9001 : 2008).

In specific research projects, accessions of the most important species like *Lolium perenne* L. or *Poa pratensis* L. are described for traits concerning morphology and/or relevant for breeding in order to have an assessment on the value of PGR for different uses.

In an overview, we will present which tasks have to be fulfilled and how this is achieved. Some results of characterization/evaluation trials and of specific research projects are provided, setting the stage for a better use of forage genetic resources in Germany, Europe and world-wide.

References:

Boller, B., E. Willner, L. Maggioni and E. Lipman, compilers. 2005. Report of a Working Group on Forages. Eighth meeting, 10-12 April 2003, Linz, Austria. International Plant Genetic Resources Institute, Rome, Italy. pp. 11, 112, 184.

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Characterization of alfalfa *(Medicago sativa)* genetic resources for breeding purpose

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Abstract

Genus *Medicago* contains about 50 species, majority of them are either diploid (2n=16) annuals or tetraploid (2n=32) perennials. *Medicago sativa* complex includes two subspecies growing in Latvia – *M. sativa* subsp. *sativa* and *M. sativa* subsp. *falcata*. Diploid and tetraploid forms exist in both subspecies. If plants have the same ploidy level, crosses of subsp. *falcata* and *sativa* produce viable hybrids (*M. varia*) (Brummer, 2004). Both subspecies are cultivated as forage crops but could also be found growing naturally. The field evaluation and characterization of alfalfa samples is performed in the Research Institute of Agriculture, Latvia University of Agriculture, genetic characterization, in their turn, is carried out in the Institute of Biology, University of Latvia. Common expeditions for collecting samples of naturally growing alfalfa populations are organized by both institutes. Ploidy level of accessions of Latvian alfalfa genetic resources, including semi-wild populations, accessions repatriated from the N. Vavilov All-Russian Institute of Plant Industry (VIR), and commercial varieties, were investigated. To determinate possible gene flow plants of subsp. *sativa* and *falcata* were crossed. Variability of ploidy level between and within different alfalfa accessions of the Latvian origin was investigated using both flow cytometry and root tip chromosome count. Most of accessions were tetraploid (commercial varieties, breeder lines). Three accessions had mixoploid plants (a commercial variety with wide genetic background and two semi-wild populations). Crossing between subspecies *sativa* and *falcata* give viable seeds. Differences between reciprocal crosses were observed: fertility was higher if as mother plants were used plants of subsp. *sativa*. Self-pollination of used accessions ranged from 1.2 to 35.8%. Study of genetic diversity is necessary to understand ecological adaptation of natural plant populations and to estimate of it potential for breeding. For this purpose twenty nine specific retrotransposon-based PCR primers (Kalender, 2010) were tested to find out those showing highest level of polymorphism in different alfalfa samples. Three most effective primers were selected for analysis of Latvian alfalfa genetic resources, including both breeding varieties and semi-wild populations. Number of polymorphic loci differed among accessions and range from 97 till 140. Some alleles were detected only in one of investigated populations.

References:

Brummer E.C., 2004. Genomics research in alfalfa, *Medicago sativa* L. In: Wilson R.F., Stalker H.T., Brummer E.C. (eds.). Legume Crop Genomics. AOCS Press, USA, pp. 110-142.
Kalendar R, Antonius K, Smýkal P, Schulman A. 2010. iPBS: a universal method for DNA fingerprinting and retrotransposon isolation. Theoretical and Applied Genetics, 121(8):1419–1430.

Characterization of genetic diversity for resistance and quality traits using molecular tools

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Abstract

Several decades of molecular studies in forage crops like *Lolium* revealed to some extent the genetic control of traits such as crown rust resistance, flowering time, vernalisation response, and water soluble carbohydrates. The first studies focussed on only a very limited genetic diversity, up to a dozen of alleles in a series of diverse linkage mapping populations, and identified major genes or QTLs with a major effect. In more complex traits such as dry matter yield and fodder quality where multiple physiological processes contribute to the phenotype, a greater number of genes are involved and the environment often has a masking effect on the genetic component of the trait. Unraveling the genetic control of these complex traits requires the use of populations with more genetic diversity and with shorter LD. It further requires the development of high resolution phenotyping methods and molecular tools to discover and screen a wider range of genetic diversity. The different research strategies used in these highly heterozygous cross-pollinating species shifted from constructing mapping populations with a very restricted number of alleles towards association mapping in a diverse collection of germplasm, or linkage mapping in highly structured and interlinked populations. With recent developments in high-throughput genotyping, phenotyping and advanced statistical approaches for highly heterozygous and genetic diverse species such as *Lolium* more genetic diversity can be screened and exploited to understand the genetic control of agronomic important complex traits. This shift towards high resolution molecular studies on complex traits using wide genetic diversity, opens new strategies for innovative breeding in forage crops.

Association mapping approaches for characterizing complex traits in perennial ryegrass

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Abstract

Knowledge on Linkage Disequilibrium (LD) is important when considering genotyping strategies for association mapping and genomic selection. A rapid decay of LD necessitates the need for a higher marker density to enable markers to capture the entire phenotypic variation in the population. Outbreeding species with large effective population sizes are expected to have very low levels of LD. We have utilized a draft assembly of the perennial ryegrass genome to assess the extent of genome-wide LD in a perennial ryegrass association population of forage and turf types. The population was genotyped using a genotyping-by-sequencing (GBS) approach and sequence tags were mapped onto scaffolds from the draft genome assembly in order to identify SNPs. The genotypes were determined for each individual at these SNP sites, and used to calculate the squared correlation of allele frequencies between SNP pairs falling within the same genomic scaffold. The LD decayed rapidly between inter-SNP distances of 1-4Kb. The population was then used to find alleles differentiated between forage and turf types. As an example, variation in a gene involved in sterol synthesis, a precursor in brassinosteroid hormone synthesis, was identified.

GBS has so far been reported only for genotyping of single individuals. However, there are many applications where resolving allele frequencies within populations on a genome-wide scale would be very powerful, examples include the breeding of outbreeding species, varietal protection in outbreeding species, and monitoring changes in population allele frequencies. We have tested the potential to use GBS to evaluate allele frequencies within populations in perennial ryegrass. The resulting profiles have been termed Genome Wide Allele Frequency Fingerprints (GWAFFs), and we have shown how these fingerprints can be used to distinguish between plant populations. GWAFFs should find many applications, from varietal development in outbreeding species right through to playing a role in protecting plant breeders' rights.

There has been limited success to date in applying molecular markers in breeding programs for outbreeding species such as perennial ryegrass, but the availability of the sequenced genome, next-generation sequencing technologies and new genotyping strategies means that applying molecular markers for marker-assisted selection and genomic selection now is feasible.

Young researcher presentation

A breeding program started fromcontrasting genotypesof alfalfa (*Medicago sativa* L.), as characterized in the frame of the PERMED project

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Abstract

The European research project PERMED (Improvement of native perennial forage plants to enhance sustainability of Mediterranean farming systems; 2004– 2008) has run an extended and accurate characterization of alfalfa cultivars of different origin in the Mediterranean region, in order to study the genetic variability available for breeding programs (*Annicchiarico et al., 2011*).Two of the cultivars characterizedfor their performance in the Mediterranean environment, of different geographic origin and divergent adaptation, were chosen as parental lines for a breeding program started by Società Produttori Sementi in 2009: the Italian variety Prosementi and the Moroccan landrace Demnat 203. Prosementi is semi-dormant (Fall Dormancy Index 6) and winter hardy, with a wide adaptation to different environments and a high and constant yield in rainfed condition; Demnat 203 is non-dormant (FDI 11), with a specific adaptation to frequent cuts in warm irrigated condition (oasis) (*Pecetti et al. 2008*).Aim of the breeding program is the development of new genotypes with FDI in the range from 7 to 10, a good winter hardiness, tolerance to frequent cuts and wide adaptation, to be employed in areas ranging from the temperate to the South-Mediterranean climate. The breeding scheme was based on open pollinated crossing in spatial isolation of 70 parent plants for each cultivar and on subsequent half-sib selection with progeny testing. Three advanced lines deriving from this breeding program (PSB QM 4, 5, 6) are now under evaluation in field trials located in two different Italian areas (Ravenna, in the North-East Italy,44°25'4"44 N/12°11'58"92 E; Cagliari, in Sardinia, 39°13'24"96 N/09°7'5"16 E), in the framework of the research projects “High quality alfalfa for the dairy chain”, one lead by Società Produttori Sementi (funded by Regione Emilia Romagna and Fondazione Carisbo) and one lead by AGRIS. The commercial varieties and the advanced lines included in the field trials are compared not only for yield, but also for morphological and physiological traits and for several parameters related to forage quality.

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Variability of alsike clover (*Trifolium hybridum* L.) natural populations from Serbia

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Abstract

The aim of this paper is to examine the variability of natural populations of alsike clover (*Trifolium hybridum* L.) from Serbia. The study included six populations of different geographic origin. Collected material was investigated on the fields of the Institute for forage crops, Kruševac, in the period of 2009-2011. Morphological traits of individual plants: height, weight, number of tillers, leaf length were analyzed on 15 plants per population, and forage quality traits: crude protein, crude fiber and fat were analyzed in the average sample per population. The highest values of observed morphological parameters were recorded in populations IS037 (755g green mass and plant height 86.6 cm, with a 100% survival rate in the second year), IS077 (52.2 shoots per plant and also 100% survival) and IS086 (2.97cm leaf length rate and 75% survival). The mass proved to be the most variable trait, where the CV ranged from 32% to 103%. The importance of the studied traits was estimated using the method of principal components analysis. When it comes to quality traits, crude protein values ranged from 18.64% in CS072 to 20.47% in IS059, crude fiber from 17.78% in CS072 to 24.71% in RO100 and fat ranged from1.91% in CS072 to 2.51% in IS086.

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Genetic response to climate scenarios within *Dactylis* and *Festuca* of temperate vs Mediterranean origin

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Abstract

Under increasing climate change and global warming, sustainability of grasslands is of special concern since they provide numerous ecosystem services. The adaptation of forage crops to increasing droughts and heat waves raises new questions in genetics and breeding such as (1) the availability of suitable genetic resources within present cultivated forage species and cultivars; (2) the effect of climate change to induce short term genetic response within populations in relationship with change of production and persistence over time; and ultimately, the identification of plant traits to select for more tolerant grasslands. This study aimed to analyse the genetic response of temperate vs Mediterranean non summer dormant cultivars of *Dactylis* and *Festuca* after two years under contrasting climatic scenarios. In a previous experimentation, two cultivars of *D. glomerata* and *F. arundinacea* of each origin were cultivated in Lusignan (temperate site) and in Montpellier (Mediterranean site) under 4 climate scenarios by increasing summer water deficit and by applying, or not, artificial heat waves in July. In Spring of year 3, the 4 species x origin combinations under scenario S1 and S2 (-381 mm vs -492 mm in Lusignan and -530 mm vs -626 mm in Montpellier (resp.) of averaged Spring to Summer water deficit (P-ET°)) were seed-harvested in each site under pollen isolation conditions. The 16 resulting new populations and the 4 initially sown cultivars were then compared in a nursery of spaced plants in the temperate site to detect correlative genetic response to previous water deficit scenarios on phenology, persistency and water use traits. In parallel, leaf elongation and plant size were measured in greenhouse 3-months long after seed germination under optimal water conditions. The first results indicate contrasting responses across cultivars within species and across sites where climate scenarios were applied. Generally, increased water deficit under the S2 scenario did not induce larger responses than under the S1 control scenario. Phenology traits like reproductive earliness associated with drought escape were found more responsive than leaf growth traits within *Dactylis*, in contrast with tall fescue for which water deficit tolerance is mainly associated with a deep rooting system and a correlative aerial growth. However, response to water stress seemed also to prioritize the traits within species on a functional way *i.e.* to efficiently avoid water deficit and, possibly, in relationship with the residual genetic variance within cultivar. Under relatively moderate water stress in Lusignan, the temperate *Dactylis Cv* shifted towards plants with greater growing rate while it shifted towards plants of earlier flowering date in Montpellier. Conversely, under the severe conditions in Montpellier, it was the Mediterranean *Cv* of *Dactylis* only, already of early flowering date, which gave a significant response but through plant size reduction. To explore these results further, all derived populations were individually *DNA* extracted for genetic differentiation evaluation. F_{st} of 0.226 between the temperate vs Mediterranean *Cv* of *Dactylis* as well as mean genetic variance within *Cv* of 0.148, from 120 markers selected among 1536, appear promising for accurate detection of genetic response by using a recently developed *DArT* resource of more than 7600 markers overall.

This still ongoing work is supported by the French Ministry of Agriculture (*CTPS*) and contributes to the *CLIMAGIE* multidisciplinary INRA project.

Monitoring of vegetation changes in selected sinkholes in the Moravian karst, Czech Republic

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Abstract

Sinkholes are very important phenomenon increasing the diversity in karst landscape. Twenty sinkholes in the area of Moravian karst have been monitored since 2000. Plant recording and phytosociological releves have been performed twice a year. The sinkholes are located at karst plateau, with intensive agriculture, resulting in very intensive erosion and underground water eutrophisation and pollution. That is why the grassland belts were established along most of the sinkholes in order to decrease the level of erosion and eutrophisation. It was partly successful, but especially in the central part of the sinkholes ruderal plant species prevail. The most predominant ruderal species are *Urtica dioica*, *Agropyron repens* and *Cirsium arvense.* After using total herbicide and sowing a special seed mixture in the year 2010 the situation improved significantly. Total number of plant species before the treatment varied from 19 to 86, the actual numbers are from 40 to 84 per sinkhole. Basing on the results of the monitoring, management proposals were prepared and applied. The most important management practices are cutting 2-3 times per year, removing the hay from the sinkhole, keeping the grassland belt along the sinkhole and planting groups of native small trees and shrubs in order to form natural formations for animals.

Evaluation of a diverse collection of red clover germplasm for susceptibility to clover rot (*Sclerotinia trifoliorum*) and other important traits

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Abstract

Clover rot, an important disease in European red clover crops, is caused by *Sclerotinia trifoliorum* or *Sclerotinia sclerotiorum*. Until today, little is known about the variation in aggressiveness among *Sclerotinia* isolates from red clover. Red clover cultivars are known to differ in susceptibility to clover rot, but few previous studies have phenotyped diverse red clover collections. It is unclear whether cultivars are generally more susceptible than wild accessions or landraces. Moreover, the relations between susceptibility to clover rot and other diseases have never been studied before. In this research we compared the aggressiveness of 30 *Sclerotinia* isolates from 25 locations in Europe. Aggressiveness was assessed in vitro on detached leaves from five genotypes and in a greenhouse on young plants from five cultivars. In a second part, we evaluated a diverse collection of 122 red clover accessions on the field. Susceptibility to mildew (*Erysiphe polygoni*) and rust disease (*Uromyces trifolii*) were scored visually on the field, along with other important traits such as plant yield, crown branching and flowering date. A similar series of accessions were scored for clover rot susceptibility in the greenhouse by inoculating young plants with ascospores from five aggressive *Sclerotinia* isolates. The susceptibility of cultivars was compared with wild accessions and landraces, and diploid and tetraploid accessions were compared. Correlations were estimated between susceptibility to clover rot and the other scored traits. Our 30 isolates differed significantly in aggressiveness. The most aggressive isolate were selected to screen our collection of red clover germplasm. Accessions differed in susceptibility to clover rot but no accession proved completely resistant. Diploid accessions were on average more susceptible than tetraploid accessions and cultivars were on average more resistant than wild accessions or landraces. Resistance to clover rot was negatively correlated with plant yield and resistance to rust disease. More resistant accessions can be used as starting material for resistance breeding, yet plant yield and susceptibility to rust disease should be carefully monitored when breeding for clover rot resistance.

Interspecific hybrids Trifolium pratense x Trifolium medium as the source of new diversity

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Poster No. 1

Abstract

Red clover (Trifolium pratense) is very important forage crop from the leguminous family where already three species have been sequenced. It is an allogamous diploid (2n = 2x = 14) with a 418 Mb genome. T. pratense is a high-quality fodder crop but shows a low persistency, which may be overcome by hybridisation with species that produce rhizomes. Hybrids between T. pratense (2n = 4x = 28) and T. medium 2n = 8x = 64) have been previously obtained by embryo rescue. The aim of this study was to evaluate interspecific variability of hybrids on the cytological level via flow cytometry and genomic in situ hybridization and on the phenotypic level via 16 characteristics.

Plants of 99 families in two breeding nurseries had comparable chromosome numbers in five groups: 2n = 28 (76 and 66% of plants), bellow of 28 chromosomes (8.5 and 6.4% of plants), 2n = 29, 30 (15 and 26% of plants), 2n = 31, 40 (0.4 and 1.2% of plants) and 2n = 41 to 46 (0.1 and 0.3% of plants). The identification of introduced chromosomes or translocated parts was unsuccessful. The phenotypic characteristics were evaluated in 500, 745 and 112 hybrid plants in three years. There were significant differences between the hybrids and T. pratense in nearly all of the analysed characteristics. The stem number per plant was significantly higher in the hybrids compared to both parental species, which could have a positive impact on the yield. Short rhizomes were observed in the hybrids after the harvest of the plants in the second harvest year. This trait will be studied from the viewpoint of improved persistency. The variability useful in breeding was significantly increased in resulting genotypes, therefore they were used as new breeding material and new variety PRAMEDI has been applied for grant of plant variety rights for testing in Central Institute for Supervising and Testing in Agriculture. In addition, interspecific variability will be evaluated by genomic procedures based on de novo sequencing of the both parental genomes by Illumina. DNA marker typing systems have been developed for genome-wide comparative analyses and association mapping of candidate genes.

The financial support of the Ministry of Agriculture of the Czech Republic (grant no. QI111A019) is acknowledged.

Factors influencing preference of tall fescue genotypes for grazing sheep

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Poster No. 2

Abstract

Tall fescue is a very robust grass species with a high yield potential. A major limitation for the use of tall fescue is the lower palatability, resulting in lower performances of cattle grazing tall fescue, compared to ryegrasses. The best measurement of palatability is done by animals themselves, but the use of grazing animals in large breeding programmes is not easy. As soft leaved genotypes proved to have a higher palatability in the past, the softness of the leaf blades, determined by touching the plants, is used in practical breeding to select genotypes with a higher palatability. Scoring for leaf softness is highly subjective and demands much of experience, moreover leaf softness is a very complex measure influenced by other factor like leaf dimensions, regrowth speed, plant architecture…. Repeatable, stable and quantifiable measures that can be linked to palatability could ease the breeding for more palatable genotypes of tall fescue.

We established a trial to find relationships between the grazing preference of animals and other plant measurements. Sixteen different clones were selected from a breeding programme and swards of 2m² were planted in four replications for each clone. On four different occasions through the year 2012, sheep were allowed to graze the swards. Grazing preference was determined by visual assessment and by pre and post grazing height measurements. Prior to the grazing allowance different measurements were made: length, width and shear strength of the leaf blades, dry matter content of the harvested material and digestibility of the organic matter . In addition, scores were given for leaf softness, leaf colour and rust infection. Using multivariate statistics the factors with the greatest effect on animal preference were determined. Our first results are suggesting that apart from leaf softness, dark colour and leaf blade shear strength are the parameters most influencing the preference by animals.

Genetic diversity of red clover (*Trifolium pratense* L.) determined by phenotypic traits and SSR molecular markers

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Poster No. 3

Abstract

Red clover (*Trifolium pratense* L.) is the only species of section *Trifolium*, sub-section *Trifolium* with a basic chromosome number n = 7, while other species in this and other closely related sub-sections (*Intermedia, Alpestre*) have a basic number of chromosomes, n = 8, which complicates interspecies hybridization. Within species *Trifolium pratense* L. there are a wide array of wild populations, which represent a valuable material for breeding. In contrast to the huge variation that exists in the germplasm of red clover, development and planting of improved varieties in recent years has resulted in a decrease in the number of local ecotypes and wild forms of red clover. Each breeding program should primarily be based on the selection of existing varieties and local populations. The aim of this study is to analyze the genetic polymorphism of 87 red clover varieties by phenotypic traits and SSR molecular markers. The experimental part of the trial was carried out at the Experiment Field of the Forage Crops Department of the Institute of Field and Vegetable Crops in Novi Sad. In the spring of 2011, a trial with 87 red clover accessions in three replicates was set up using a randomized block design. Each red clover accession (genotype) was represented by a sample consisting of 30 plants (10 plants per replication). The row-to-row spacing was 80x80 cm. At the phenological stage of the beginning of flowering (in mid-July) the following traits were studied: stem length (cm), stem thickness (mm), number of internodes, length of central leaflet (mm), central lamina thickness (mm), time of flowering, growth habit, density of hairs on the stem, intensity of spots (white marks) on the leaf, yield of green mass and dry matter yield. Compared to other molecular markers, microsatellites show a much higher level of informativeness for studying the genome of red clover. Cluster analysis based on the phenotypic traits and SSR molecular markers will reveal genetic diversity in this part of red clover collection on both of levels of investigation.

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The utilisation of wild *Fabaceae* species in grasslands

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Poster No. 4

Abstract

The possibility of selected wild *Fabaceae* species cultivation as grassland components was tested in the period2004-2010 in the trials performed in Troubsko. The field experiment was established with the use of standard grass mixture to which 50 seeds of tested species were added. The standard grass mixture contained these species: *Festuca pratensis*, *Lolium perenne*, *Poa pratensis*, *Phleum pratense* and *Festulolium*. Twenty species of the folowing genera were tested: *Trifolium*, *Vicia*, *Lotus*, *Lupinus*, *Lathyrus*, *Astragalus*, *Melilotus*, *Medicago*. The experiment was established by the method of randomised blocks in three repetitions. The size of one plot was 5 m^2. The stand was harvested two times per year. From all observations of wild species in grass mixture the average numbers of plants were calculated. The soil-climatic conditions of the locality were the limiting factors for the survival of tested species.

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Characterization of some local populations of *Medicago minima* collected in Central Steppe of Algeria

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Poster No. 5

Abstract:

The aim of our study is to evaluate some local populations of *Medicago minima*coming from 2004 prospection conducted by INRA Algeria through all the Djelfa area (Central Steppe). The study is based on quantitative characters of plant, pod and seed. The results obtained show that there is high genetic variability within populations of *M.minima*. Analysis of variance showed highly significant differences between populations for all parameters studied. We noted that the early flowering stage setting was the most discriminating character. For results of correlations, several correlations were observed between various parameters studied. We have noted correlations between various traits related to pods and seeds, but we noted no correlations between vegetative development traits.

Breeding perennial ryegrass with enhanced water soluble carbohydrate content

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Poster No. 6

Abstract:

A high water soluble carbohydrate (WSC) content in perennial ryegrass leads to a more efficient use of the protein in the grass by the ruminant and hence to reduced nitrogen losses.

In our breeding pool we search for breeding material of diploid and tetraploid perennial ryegrass with enhanced WSC content. In 2011 we have sown a yield trial in which we compare high sugar varieties with diploid and tetraploid breeding material with expected high WSC content.

Some of the material (families, syn1-populations, induced tetraploids) was not selected for a high WSC content, but showed a high WSC content in previous trials. Other material was based on crosses (pair cross, top cross, polycross) with genotypes selected for their high WSC content.

The yield trial was harvested five times in 2012. We took samples at each cut to determine the WSC content by NIRS. The results of the analyses will be presented.

Different aspects of shoot branching in red clover

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Poster No. 7

Abstract

Red clover has several advantages in agriculture: the ability to fixate nitrogen, a high seedling vigour and a high nutritional value for feed and indirectly for food (high protein and polyunsaturated fatty acid levels). However, the current red clover cultivars have a low persistence. Plant architecture, which is under genetic and environmental control, may have a strong influence on agronomic important traits such as forage yield, re-growth capacity, seed yield and persistence in red clover. We have done an in-depth study of the architecture of a limited number of red clover genotypes with contrasting branching phenotypes based on the number of branches, the outgrowth of the main axis and the growth habit (erect/prostrate). A detailed morphological analysis showed differences in the number of buds, the number of branches and the position of bud outgrowth in the different genotypes. The bud outgrowth percentage (defined as the percentage of buds that grow out into branches) was also significantly different among genotypes. Also, differences were observed in the re-growth after cutting between the genotypes. In a physiological approach, isolated single node fragments were used to study the influence of branching hormones on bud outgrowth without having to deal with the complex architecture of a complete red clover plant (non-outgrowing main axis, many first-order branches). These results were linked to expression levels of genes involved in branching according to literature. In addition, we determined concentrations of endogenous strigolactones and auxins in intact plants of the various genotypes. The results demonstrate that both bud formation and bud outgrowth are important to explain branching differences in red clover and that both the strigolactone and auxin pathways are relevant candidates for further analysis and molecular breeding for high yielding and more persistent red clover cultivars.

Fertilization value of early red clover (*Trifolium pratense* L.) 'Jõgeva 433', washington lupin (*Lupinus polyphyllus* Lind.) 'Lupi' and crimson clover (*Trifolium incarnatum* L.) as green manure crops

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Poster No. 8

Abstract

In 2008-2011, field trials were carried out at the Jõgeva Plant Breeding Institute (58°45' N, 26°24' E) in order to identify the possibilities of using the early red clover cultivar "Jõgeva 433" (diploid), Washington lupin with Jõgeva breeding cultivar 'Lupi' and crimson clover material of Italian origin as green manure. The fertilization value of the above species was studied after autumn ploughing in the year of sowing and second year from sowing based on the yield and quality of yield of spring wheat "Vinjett" and barley "Inari". The in-ploughed biomass as well as its composition were recorded. By the time of ploughing the most abundant biomass has been grown by Washington lupin 'Lupi'. The root system of the species is well developed; roots can be as thick as fingers. The sod is easily ploughable, but the roots remain alive until next spring and the recovering lupin plants may interfere with the aftercrops.

Of the studied species crimson clover had the lowest fertilization value, the stubble and roots of seed plants that were ploughed in resulted only in 6-7% of extra yield of spring wheat in the following year. Crimson clover had no fertilizing aftereffect in the second year. The fertilization values of red clover 'Jõgeva 433' and Washington lupin 'Lupi' were more or less equal. Their statistically significant positive effect on the increase of yield of spring wheat and barley lasted for 3 years, on the quality of yield for 2 years. Red clover 'Jõgeva 433' that was ploughed into ground in the year of sowing gave the maximum extra yield of 27% in spring wheat compared to N 0 variant, and Washington lupin 'Lupi' respectively 23%. In the second year of aftereffect Washington lupin 'Lupi' gave in barley an extra yield of 26.8%, red clover 'Jõgeva 433' 10.1%, and in the third year 10.4 and 12.1% respectively. Green manure improved the contents of crude protein and gluten in spring wheat and that of crude protein in barley.

The fertilization value of all studied species was higher in the case they were sown without a cover crop.

Washington lupin 'Lupi' cannot be recommended to organic farmers, because without the use of herbicides the species may become a troublesome weed.

Chloroplast proteome of *Festuca arundinacea* during drought conditions

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Poster No. 9

Abstract

Festuca arundinacea (tall fescue) is one of the most drought tolerant species within the *Lolium-Festuca* complex, and was used as a model for research aimed at identifying the chloroplast components involved in proteomic response for drought stress in forage grasses. Two *F. arundinacea* genotypes with contrasting levels of tolerance to short term drought, the high drought tolerant (HDT) and the low drought tolerant (LDT) genotype, were selected for comparative physiological and proteomic work. Measurements of water uptake, chlorophyll fluorescence, relative water content, electrolyte leakage and gas exchange during drought and re-watering periods were followed by investigations on accumulation levels of chloroplast proteins before drought conditions, on the third and 11th day of drought treatment, and after ten days of subsequent watering, using two-dimensional gel electrophoresis. The proteins which were accumulated differentially between the selected plants at least at one analyzed time point were then identified by mass spectrometry. The LDT genotype revealed lower levels of water uptake and relative water content as drought progressed, and this was accompanied by lower levels of transpiration and net photosynthesis, and higher level of electrolyte leakage observed in this genotype. Eighty two protein accumulation profiles were compared between the HDT and LDT genotype and ten proteins were shown to be differentially accumulated between them. All the identified proteins, involving Rubisco activase, cyclophilin, fibrilin, lipocalin, zinc metalloprotease FtsH2 and others, were shown to be involved directly in the photosynthetic reactions, or in the protection of photosynthetic apparatus against different components of drought stress. Moreover, the accumulation patterns of the selected proteins clearly indicated that the HDT genotype accumulated significantly higher amounts of the crucial proteins, at least at some analyzed time points of the experiment, than the LDT genotype. This supports the hypothesis that some of these proteins could be involved in regulation of drought tolerance in *F. arundinacea*.

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Heritability and genetic correlations of forage yield traits in alfalfa (*Medicago sativa* L.) for highland central valleys of Mexico

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Poster No. 10

Abstract

Highland Central Valley of Mexico includes the state of Hidalgo, Mexico, Puebla, Queretaro and Tlaxcala, and alfalfa is the fifth agro-economical crop, widely used for dairy and beef cattle and sheep feeding under harvesting or grazing conditions. However, no cultivars evaluation and breeding program exists, and our task is to screen and genetically characterize novel germplasm highly adapted, persistent and yielder (Alarcon-Zuniga, 2012). With this task, 65 alfalfa cultivars were sown in 4.5x1m plots with three replications in a rectangular lattice design. Irrigation, weed control and 60-80-40 annual fertilization were provided. Nine harvests were done and the ten high yield cultivars are reported herein. Half sib families were derived of the ten high yield cultivars, transplanted in a 10x15 rectangular lattice design with four replications, and three harvests done in the first year of establishment to estimate heritability and genetic correlations among yield traits. According with data, there was significant variation among cultivars, with cvs commonly used in Mexico were intermediate to low yield. All cultivars showed same yield pattern through the nine harvests, with high forage yield during summer and low in winter. Broad sense heritability on an HS family basis of DMP, DM ha and plant height were from intermediate to high.

Phenotypic and genetic correlations of half sib families for all ten high yield cultivars between plant height and forage yield were 0.53 and 0.411 ($p>0.001$), and were consistent for every harvest, indicating that taller plants are more productive. As expected, dry matter per plant and forage yield were genetically correlated (0.71**). These results indicate that selection of variables for the productivity could enable breeders to increase forage yield of alfalfa, both within and among populations. It was concluded that alfalfa cultivars screened for forage yield components showed a wide variability to adaptation to Central Valley of Mexico, with cvs. African and Indian Populations, Tshyuwakaba and INIA-76 can be used as parental genotypes.

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Variation in cell wall digestibility of perennial ryegrass at heading stage

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Poster No. 11

Abstract

Perennial ryegrass (*Loliumperenne*) is the dominant forage grass in Europe, because of its high protein content and energy value. Parsons et al. (2011) showed that a higher ratio of WSC (water soluble carbohydrates) to CP (crude protein)contentresults in a lower nitrogenurine excretion,indicating that the conversion of grass protein to meat or milk protein is more efficient for high-energy grasses. However, high levels of WSC lead to rumen acidosis and a reduced voluntary intake. Hence, the energy contained within the cell walls, which is released more gradually than the soluble carbohydrates, has become a focus in ryegrass breeding.

Selecting varieties with a high cell wall digestibility requires extensive knowledge of the variation available in the genepool. We have assessed the cell wall digestibility (NDFD) of a set of genotypes harvested at heading stage, i.e. the stage most relevant for agricultural application. NDFD values were estimated by NIRS for separate organs (blade, sheath and stem) and, using theirweight fractions, the NDFD of the total plant was calculated.

Significant genotype-to-genotype differences were found for total plant NDFD, be it limited: mean NDFD values varybetween 72.6% and 78.5%. For sheath and stem,the observed variation in NDFD is higher than for the total plant(from 65.4% to 77.2% and from 56.1% to 76.6% respectively).For blade, inter-genotypic differences were not significant (73.7% to 78.8%).

In addition to the NDFD value of the different organs, also the weight fractions of leaf, sheathand stem have an impact on the total plant NDFD values. Among the 13 genotypes analyzed, the weight fraction of blade varies from 47% to 88%, the sheath fraction from 7 to 33% and the stem fraction from 3% to 18%.

Studying this limited set of genotypes revealed limited variation in NDFD atthe plant level, buta significant variation in organ fractions and organ-specific NDFD values, indicating potential selection targets to improve cell wall digestibility at the whole plant level.

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Genotype, species and environment in forage pea/cereal mixtures

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Poster No. 12

Abstract

Produced forage quality is of crucial importance since the optimum dietary protein for lactating cows is about 16.5% of DM (Broderick, 2003) and overfeeding of concentrates, to compensate for low forage quality, usually has adverse effects on milk fat secretion and results in metabolic problems for the cow.

Winter forage pea offers high yielding capability with desirable crude protein levels (22.1-15.8% in pea DM, Stjepanović et al, 2008) and is usually grown in mixtures with wheat of poorer quality (13.8-10.0 crude protein in DM) to prevent lodging and increase DM yield. Genotype, species and environment largely influence the earliness, yielding capability and forage quality of winter forage pea/cereal mixtures. Early achieving desirable yields of winter forages provides for earlier establishment of subsequent forage crops (which are usually low in crude protein) thus ensuring a greater cumulative annual forage yield. Aim of the research was to investigate the pea genotype, cereal species and environmental conditions effects on the forage yield, pea partition and forage quality.

The research was conducted with pea (two opposing types of cultivars) / cereal (wheat and triticale) mixtures in east Croatia in 2010/2011 and 2011/2012 seasons. Environmental conditions greatly influenced the yields: in the late cutting terms in 2011 there were achieved yields of pea/wheat mixture from 7.05 (21st May) up to 15.67 (31st May) t/ha DM, whilst in 2012, in relatively earlier cutting terms, there were achieved yields of pea/wheat mixtures up to 5.75 t/ha DM (18th May) and of pea/triticale mixtures up to 7.55 t/ha DM (18th May). Research has revealed that new bred forage pea cultivar Letin combined trait of early development and afila leaf trait, so providing higher yields in early terms, greater pea partition in the yield and improved lodging resistance compared to conventional cultivar Osječki zeleni. Triticale has shown faster development, greater DM yields and lower pea partition in mixtures compared to wheat. The pure pea mixture of two investigated cultivars has shown satisfactory lodging resistance and relatively high DM yield of 6.59 t/ha (18th May). Quality of mixtures was strongly related to the pea partition in the yield.

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Variability of selected traits in the Czech alfalfa core collection

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Poster No. 13

Abstract

Within the set of 457 origins (varieties, newly bred varieties and wild forms) of alfalfa (*Medicago sativa*, *Medicago* x *varia* and *Medicago falcata*) of the world collection maintained in the Czech national gene bank 52 characters were evaluated. The traits were found out on individual plants grown in the field outplanting. From the obtained mean values of particular weighted, measured and counted characters the estimations of correlation coefficients were calculated in 28 of them. The estimations were used as the extent of the tightness of linkage of measured traits. The obtained results are presented in the matrix and are commented. Furthermore, for each origin and 22 selected characters the variation coefficients were estimated from the observed values. These coefficients were used as the measure of within-varieties variability. The biggest average variability reached the following traits: the weight of seeds per tuft, number of inflorescences per stem and the number of lateral branches. The lowest average variability reached the following traits: the height of the growth in the beginning of flowering, the length and the width of the terminal leaflet and the stem diameter. The origins with the biggest and the lowest values of the variation coefficients of each trait were chosen.

Drought effect on yield of perennial ryegrass (*Lolium perenne* L.)

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Poster No. 14

Abstract

Breeding for drought resistance forms a major challenge for plant breeding and gains now more interest in Europe, because drought periods are expected to increase in the 21st century as a result of climate change (Alcamo *et al.*, 2007).

During late summer 2009, late spring 2010 and early spring 2011, three differentdrought periods in Belgium caused severe reductions in dry matter production of the corresponding cuts in our fodder grass field plot trials. Yield inhibition was most spectacular when the drought period occurred in early spring (April).

Dry matter production was clearly inhibited for perennial ryegrass (*Lolium perenne* L.) genotypes, while tall fescue (*Festuca arundinacea* Schreb.) performed better during the drought periods.

Significant positive correlations were found when the dry matter yield of different perennial ryegrass genotypes was compared between the three drought periods. These correlations and a considerable variation in response to drought within these genotypes show potential to improve drought resistance in perennial ryegrass.

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Stability analysis in foxtail millet landraces, pure lines and composite cultivars for forage yield

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Poster No. 15

Abstract

Foxtail millet [*Setariaitalica* (L.) P. Beauv.] is a C_4 summer annual cropcultivated in subtropical and temperate regions all over the world. It's a short-cyclecrop with high water-use efficiency,making it suitablefor low-rainfall areas and semi-arid regions. Foxtail millet is grown in Argentina as a fodder crop for whole-plant hay and silage in beef and milk production systems. Forage yield stability of foxtail millet cultivars is important to enhance flexibility in crop rotations and stabilize farmer incomes. Genetic heterogeneitymay be an option to improve cultivar's buffering capacity against random environmental factors (Smithson and Lenné, 1996).In the present study, forage yield potential and stability ina set of genotypes was evaluated across 26 environments from 2006 to 2012. These genotypes included three cultivar types: traditional landraceswith broad and narrow genetic bases (Velazco and Rimieri, 2012) released in the 1960s (LR),elite pure linesisolated from these landraces (EL), and composite cultivars (CC) derived from each landrace(mixtures between 6 and 17 pure lines). Both, EL and CCare originating from the breeding program at INTAPergamino Experimental Station since 2006.A mixed model approach was applied for dataanalysis to handle with spatial variation within trials, error variance heterogeneity between trials and complex GxE structures (Smith *et al*. 2005). This flexible framework allows for reliable estimation of genotypic values and for modeling specific cultivars' variability across environments.The highest yielding cultivars were EL and CC. However, there was not a clear associationbetween cultivar typesand stability levels.The analysis showed that there are some pure lines and composite cultivars outperforming traditional landraces in broad adaptation and static stability stressing the importance of intra-plant buffering capacity. This study provided information to identify superior cultivars for further breeding testing and recommendation to farmers based on overall performance in the target population of environments. Furthermore, with this multi-environment evaluation it was able to compare historical and modernfoxtail millet cultivars under the same agronomic practices and to obtain a fairly realistic measure of the genetic gain in forage yield over 50 years.

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Morphological variation between perennial ryegrass (*Lolium perenne* L.) wild ecotypes

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Poster No. 16

Abstract

Perennial ryegrass (*Lolium perenne*) is highly important forage and turf grass. A new variety must be not only stable and uniform, but also distinct from other varieties. Therefore variability of phenotypical traits is very important in breeding.

The objective of this study was to evaluate diversity of morphological traits of wild perennial ryegrass ecotypes. Wild ecotypes from Lithuania (5), Poland (2), Russia–Kaliningrad (3), Slovakia (1) and Ukraine (40) were planted in 2011–2012 into the germplasm collection at Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry. Winter survival (%), re-growth in spring (point), plant height (cm), beginning of heading (date), flag leaf length and width (cm) and dry matter yield of 1st cut (g per plant) were assessed.

High variation of re-growth in spring and biomass (CV=22.69 and 22.38 %) and low – of winter survival and plant height (CV=6.07 and 8.11) was detected among ecotypes. The average (CV=10.57–16.39 %) polymorphism between wild ecotypes was established for other traits. Principal component analysis was carried out and a dendrogram was computed based on morphological traits. In the grouping of ecotypes were mostly influenced by plant height, flag leaf length and width, dry matter yield and date of heading.

The paper presents research findings, which have been obtained through long-term research programme "Genetics and purposeful change of genotypes of agricultural and forest plants" implemented by Lithuanian Research Centre for Agriculture and Forestry. Wild ecotype collection expenditures in Ukraine were covered through Lithuanian – Ukrainian bilateral research cooperation programme (TAP-LU-11-027).

Indicating soil quality using urease and saccharase activity in abandoned grassland and differently managed crop fields

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Poster No. 17

Abstract

In order to ascertain and make a comparison of bioactivity variation during vegetation period, soil (*Hapli–EpihypogleyicLuvisol (LVg-p-w-ha), Albi–EpihypogleyicLuvisol (LVg-p-w-ab), and Hapli–AlbicLuvisol (LVA-Ha)*samples collected in fields of different fertilizing and farming systems: extensive (ExF), conventional (CF) and organic (OF).The aim of this study was to determine the saccharase and urease activityresponses to different land management systems and main soil quality indices such as soil genesis type, content of soil organic carbon (SOC), total nitrogen (N), and C/N ratio. The trial was comprised of eight experimental plots (three land management systems and two crops groups: legumes and gramineous).

Assessed hydrolases were observed as sensitivity-able indicators suitable to evaluate parameters of soil quality. The highest decrease and the lowest rates of urease (1.13 mg NH_4^+–N g^{-1}) and saccharase (8.40 mg CG g^{-1} 24 h^{-1}) activities were observed in abandoned grassland soil where mineral fertilizers were not applied.Enzyme activity correlated stronger with SOC (r=0.7) or ratio C/N (r=0.6) than that with total nitrogen content (r=0.5-0.6). Significantly higher 3 year mean value of saccharase (27.00 and 12.6 mg CG g^{-1}) and urease (5.78 and 4.16 mg $NH_4{}^+$–Ng^{-1} 24 h^{-1}) were observed insoil under conventional and organic management in compare with abandoned grassland. Consequently, it can be concluded that enzyme activities might be responsible to the different level of soil fertility.

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Patterns of morphophysiological diversity and their implications for selection of grazing-tolerant lucerne

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Poster No. 18

Abstract

Grazing-tolerant lucerne has gained popularity as a component of legume-grass binary mixtures in grazing systems of temperate regions. A major challenge for lucerne breeders is combining high plant persistence under continuous grazing with high forage yield in a mown harvest of spring, the season in which the excess of forage production for the grazing animals can conveniently be exploited for conservation. An additional challenge for breeders targeting mild-winter regions is the selection of grazing-tolerant material with good autumn and early spring growth (i.e., low autumn dormancy), because grazing-tolerant genetic resources tend to possess high autumn dormancy as a consequence of substantial introgression from ssp. *falcata* germplasm and the specific adaptation to cold-prone environments of this subspecies (Pecetti et al., 2008). Various studies suggests the presence of a genetically-based trade-off between persistence under continuous grazing and forage yield under mowing (Smith et al., 2000; Pecetti et al., 2008), indicating that these production traits are maximized by contrasting morphophysiological types (the prostrate type tending towards high grazing tolerance but low forage yielding ability). It is unclear whether the relationship of persistence under grazing with autumn dormancy is genetically based. The objectives of this work are (i) exploring the patterns of morphophysiological diversity in each of four biparental populations originated by crosses between prostrate and erect parent germplasm, and (ii) assessing their genetic relationships to grazing tolerance (estimated as plant survival after one year of heavily-stocked continuous sheep grazing), forage yield in two mown spring harvests preceding the grazing season, and autumn and early spring vegetation. Each population included over 100 F_1 progenies and their parent genotypes evaluated as clonal material (four clones per plot) in a randomized complete block experiment with three replications. The results from this study can help understand to which extent different useful agronomic traits can be combined into a grazing-tolerant variety with wide flexibility of utilization (continuous sheep grazing possibly combined with one mown spring harvest) and adaptation to the relatively mild-winter environments that feature most south-European cropping regions.

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The variability of white clover (*Trifolium repens* L.) wild populations from Serbia for the main agronomic traits

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Poster No. 19

Abstract

This paper presents the results of three-year pre-selection research of 11 white clover wild populations. They were collected on Serbian mountains from habitats with different altitude. The study was performed on spaced plants nursery sown on 1x1m distance. Each population was presented with 30 plants in three replications. Following traits were measured in three years period on all plants and presented as average values: plant height, leaf length, leaf width, width of plant sward and dry matter yield per plant. In second year of trial crude protein content, neutral detergent fibre, acid detergent fibre and acid detergent lignin were determined. The results were analysed by ANOVA and there were statistically significant differences between investigated populations for the most of studied traits. The highest inter population differences were found for dry matter yield, size of leaf and width of plant sward. Variability of individual plants in all populations for all investigated morphological traits, except plant high, were high. Data were analysed by cluster analysis using Ward method and Euclid distances. The cluster diagram based on all studied traits shows three groups of investigated populations.

Intensive management reduces genetic diversity of *Dactylis glomerata* in Swiss permanent grassland

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Poster No. 20

Abstract

Genetic diversity, i.e. the diversity within species and populations, is an important component of biodiversity which may substantially contribute to the productivity, adaptability and sustainability of grassland ecosystems. Although the importance of grassland habitats as a reservoir of genetic resources is generally recognized, little is known about the effect of environmental factors and agricultural land use on genotypic and phenotypic diversity.

This study aimed to investigate orchard grass (*Dactylis glomerata*), a perennial forage grass well adapted to a broad range of temperate environments as a model species to assess the effect of management practices (intensive, extensive) and farming systems (organic, non-organic) on genotypic and phenotypic diversity

Twenty orchard grass populations were sampled from alpine, permanent grassland habitats, contrasting in respect to organic and conventional farming systems, management intensity and environmental conditions. Genetic diversity was analyzed using 29 simple sequence repeat markers (SSRs) and 10 morpho-physiological traits evaluated in a field experiment.

Nei's unbiased gene diversity (H_E) revealed a high genetic diversity (0.50 – 0.55). Most of the genetic variation resided within populations (97.9%). Genetic diversity was significantly influenced by intensification of management resulting in a decrease of genotype diversity within sites ($r = -0.50$, $p = 0.02$). Furthermore, phenotype evaluation revealed significant influence of management on *Dactylis glomerata* populations based on selected traits. However, within- and between-population genotypic and phenotypic diversity was marginally correlated with faming systems.

Facing uncertain climatic changes and an increasing need for sustainable management of resources, the insights gained may be particularly valuable for the *in situ* conservation of valuable genetic resources of grassland species. Furthermore, they support the improvement and targeted selection of ecotypes for breeding programs.

Agro-morphological and forage quality traits of selected alfalfa populations and their application in breeding

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Poster No. 21

Abstract

The objectives of this research were to evaluate agro-morphological and forage quality performances of selected alfalfa populations and to predict the genetic potential of this populations as a source of the material for breeding programs and/or commercial use. Twenty superior alfalfa populations (MSP1 to MSP20) and two control cultivars (OS-88, OS-99) were tested over three consecutive years (2008, 2009, 2010) at the experimental field of the Agricultural Institute Osijek in Croatia. The experimental design was a randomized complete block with three replications. The plot size was 1x6 m at the sowing rate of 15 kg ha^{-1}. A total of fifteen traits (yields of green mass and dry matter, crude protein yield, plant height, plant regeneration, number and length of internodes, stem thickness, width and length of central leaflet, leaf to stem ratio, contents of crude protein, neutral and acid detergent fiber in dry matter, relative feed value) were measured/analysed on all plots and/or individual plants of all populations and cultivars. All collected data were processed by analyses of variance applying the least significant differences test using the general linear model procedure of SAS software. Significant differences were determined between alfalfa populations/cultivars in all investigated traits, except for content of crude protein in dry matter. The highest three-years average yields (from 86.32 to 101.12 t ha^{-1} green mass, from 17.45 to 20.46 t ha^{-1} dry matter, from 3657 to 4289 kg ha^{-1} crude protein) and favourable values for the most of the investigated morphological and forage quality traits were obtained at populations MSP 16,8,1,9,20,11,10. Seven superior new developed alfalfa populations were identified which could represent a valuable source of genetic material for further selection and improvement of our breeding program and/or application for plant cultivar registration.

This research was supported by the Ministry of Science, Education and Sport of the Republic of Croatia (“Evaluation of breeding value in alfalfa (*Medicago* spp.) germplasm – No. 073-0000000-3535) and bilateral project (Croatia-Slovenia) “Importance and role of forage legumes in sustainable agriculture and in the environment preservation“.

New insights into alfalfa forage quality through the research project Qual&Medica

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Poster No. 22

Abstract

The research project "High quality alfalfa for the dairy chain" (short name Qual&Medica), led by Società Produttori Sementi and financed by Fondazione Cassa di Risparmio of Bologna and Regione Emilia-Romagna, focuses on management and genetic tools to improve the feeding quality of alfalfa forage for the dairy chain, in order to increase the crop profitability in hay-based farming systems. Three workpackages of the project aim at defining: (i) breeding strategies to increase the crop feeding value; (ii) mowing strategies to improve hay quality; (iii) rapid analytical tools for quality classification of harvested forages. One last workpackage has the purpose of communicating the project results and providing technical support to growers who target high quality forages. The website www.qualemedica.it hosts information on the project objectives, development and results. Qual&Medica pays specific attention to the evaluation of genetic and environmental determinants of alfalfa forage quality parameters, such as protein and fiber fractions, fiber digestibility, sugars and minerals contents, assessing their relation with morphological and physiological characteristics. The variability and heritability of quality traits are investigated in experiments under field and controlled conditions, exploiting the genotypic diversity in a wide set of varieties, landraces and advanced breeding lines. The effect of environmental and harvesting practices, and their interactions with genetic factors, are studied by applying different mowing schedules and water-stress levels on different germplasms, including innovative variety types selected for the multifoliate trait or for shorter internodes. This paper provides first results generated by project experiments carried out in agricultural and controlled-environment experiments.

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Determination of ploidy and DNA content of *Bromus catharticus* Vahl accessions by flow cytometer

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Poster No. 23

Abstract

Bromus catharticus Vahl included in *Bromus* L. section *Ceratochloa* and is native to South America. It is widely used as cool season grass for both grazing and hay in many countries. The exact ploidy levels of *B. catharticus* accessions in the USDA National Plant Germplasm System (NPGS) are unknown, which could complicate future use and breeding efforts. The objective of this study was to determine the ploidy level and DNA content of the 79 USDA NPGS *B. catharticus* accessions, and four commercial cultivars. Among the 79 accessions, 77 hexaploids and 2 octaploids were identified. The 2C Nuclear DNA content varied between 11.79 (PI 595116) pg and 13.72 pg per nucleus (PI 273869) in hexaploids. The mean 2C nuclear DNA content of octaploid accessions were 19.41 pg for PI 655178 and 19.66 pg for PI 138221. All the commercial cultivars included in the study were determined to be hexaploid.

Some aspects of breeding forage brassicas

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Poster No. 24

Abstract

Numeorus species belonging to the family *Brassicaceae* Burnett, popularly known as *brassicas*, are multi-purpose crops used not only for various industrial purposes and human consumption, but also in animal feeding. Among the most significant fodder brassicas are those that are grown for forage, such as fodder kale (*Brassica oleracea* L. var. *viridis* L.), oilseed rape (*Brassica napus* L. var. *napus*), hybrid Perko PVH (*Brassica napus* L. var. *napus* x *Brassica rapa* subsp. *chinensis* (L.) Hanelt), turnip rape (*Brassica rapa* L. subsp. *oleifera* (DC.) Metzg.) and white mustard (*Sinapis alba* L. subsp. *alba*). Those cultivated for roots comprise rutabaga (*Brassica napus* L. var. *napobrassica* (L.) Rchb.) and fodder turnip (*Brassica rapa* L. subsp. *rapa*). All of them play an important role in diverse forage crop rotations, with the autumn-sown ones much appreciated in environment-friendly production, ecological services, organic farming and sustainable agriculture. Due to their ability to produce abundant aboveground biomass, forage brassicas may also be grown as cover crops and for green manure and grazing. Among the traits with a great significance in developing new forage brassica cultivars are plant height, number of stems and lateral branches per plant, number of leaves per plant and other forage yield components, fresh forage yield and forage dry matter yield per both plant and area unit, earliness and tolerance to low temperatures, drought and other forms of both abiotic and biotic stresses. The methods of breeding forage brassicas include mass and individual selection from local landraces, usually characterised by a wide genetic variability of desirable traits, and various methods of selection from hybrid populations, with emphasis upon bulk and pedigree methods. Contemporary fodder kale breeding programmes aim at increasing leaf proportion, reaching nearly one half of fresh forage yield. Numbers of stems and lateral branches have the greatest significance in breeding oilseed rape for forage, with spring cultivars of having higher proportion of stems in fresh forage yield. In comparison to other forage brassicas, white mustard is mostly characterised by a lower leaf mass proportion in total plant mass, higher forage dry matter proportion and extremely prominent earliness.

Wild and less cultivated vetches (*Vicia* spp.) as forage crops

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Poster No. 25

Abstract

Vetches (*Vicia* spp.) are a legume genus rather rich in species and comprise several economically important crops. Faba bean (*V. faba* L.) is one of the economically most significant pulses in temperate regions, while common vetch (*V. sativa* L.) plays an important role as a both forage and grain crop in many Mediterranean climates. There are many vetch species that are still nothing but the components of wild floras or are cultivated extremely locally. In many countries of Europe, West Asia and North Africa, Hungarian vetch (*V. pannonica* Crantz) is used as both forage and grain in feeding ruminants. The available cultivars of Hungarian vetch have a sufficient tolerance to low temperatures and are characterised by a stable forage yield over the years, but heavily suffer from a prominent pod dehiscence. Hairy vetch (*V. villosa* Roth) is traditionally grown only for forage, but recently have gained an increasing importance as a cover crop and green manure, being exceptionally winter hardy and able to produce more abundant aboveground biomass than any other vetch species. It also has allelopathic effect on many weeds. The most radical advances in hairy vetch breeding are expected in identifying candidate genes controlling determinate stem growth, earliness and shorter duration of both flowering and seed maturing. Although largely grown for grain, especially in South Europe and Near east, bitter vetch (*V. ervilia* (L.) Willd.) with its morphology represent one of the possible ideotypes of forage vetches cultivars. Despite short stems, bitter vetch often produces forage yields at the same level as typical annual forage legume crops, mostly due to a great number of nodes, an excellent preservation of the majority of the leaves and an extraordinary spatial distribution of leaves in the stand volume, all resulting in a high photosynthetic activity and forage dry matter production. Another neglected vetch crop with a considerable potential for forage production is Narbonne vetch (*V. narbonensis* L.), while several wild annual species, such as large-flowered (*V. grandiflora* Scop.), French (*V. serratifolia* Jacq.) and narrow-leafed (*V. sativa* subsp. *nigra* (L.) Ehrh.) vetches, also represent potential forage crops.

Morphological evaluation of some perennial grass cultivars in Algerian semi arid conditions

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Poster No. 26

Abstract

Selection of perennial forage grasses is aimed at improving the economic and environmental sustainability of production from cattle and sheep. Tolerance to drought conditions, water use efficiency, persistence and high forage yields are the major traits that determine the adaptability of the tested genotypes. This study was conducted at the experimental site of the ITGC institute in Sétif during the cropping seasons 2005/06, 2006/07, 2007/08 and 2008/2009 with the objective of evaluating the performance of 14 varieties of perennial grasses, belonging to two species: *Festuca arundinacea* Schreb. and *Dactylis glomerata* L. in a semi arid region. Characterization has focused on various morphological traits in order to assess their adaptability to drought prone environments. The results showed the existence of a wide range of variability due to the diversity of responses of the evaluated varieties of both species, particularly regarding biomass production, production cycle, persistence and water use efficiency, which is considered as one of the most important factors in the success of artificial grasslands in semi-arid areas. These results show high potential for the selection of a plant material adapted to the specific conditions of the semi-arid areas of the Algerian high plains. Indeed, this study allowed us to discriminate the most adapted genotypes characterized by high performance, good height growth, earliness in heading and good persistence.

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Tall fescue *Palenque Plus INTA*: source of stress tolerance and forage quality in breeding program from Argentina

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Poster No. 27

Abstract

Tall fescue [*Festuca arundinacea* Schreb. (=syn *Lolium arundinaceum* (Schreb) Darbysh.)] is an important forage species in Argentina since the early 1950s. *Pergamino El Palenque MAG* (EPMAG), was the first tall fescue variety registered in Argentina and is considered the emblematic broad-based population of Argentina. It was originated from a world collection in the mid-20th century exhibited a wide genetic variability for important agronomic traits. This variety is extremely persistent and with remarkable forage production throughout the year and especially adapted at the “improved” grasslands in Pampas Region. This Region is characterized by extended croplands and native or sown temperate pastures where the most important perennial forages species are alfalfa and tall fescue. Argentinean’s varieties have adaptation to complex ambient stresses due to the important genetic variability exhibited by EPMAG variety. As a result of the above, the breeding program of tall fescue from National Institute of Agriculture Technology (INTA) registered the first synthetic variety *Palenque Plus INTA* (PPI) from forty parental genotypes chosen from EPMAG, with better adaptation and persistence, higher tillering density, a high DM-yields and tolerance to drought and stem rust (*Puccinia graminis* Pers). The remaining genetic variability in PPI is still important to applied selection. In order to obtain synthetics varieties with improved forage quality, a subsequent selection process were initiated from PPI genotypes. Two news varieties were obtained: *Brava INTA* (seven parental genotypes) and *Luján INTA* (thirty five parental genotypes). Both varieties have soft leaves, high DM-yields, drought tolerance, tolerance to effect of puddling and adaptability to soils with physic limitations. The genotypes involved certainly improved the fixation of characters associated with flexibility and quality of leaves which allowed achieving genetic progress in quality without losing productivity and hardiness. The selection advantage and improvement obtained in the new varieties is superior in 10 to 25% in DM-yields and in 3–5% in digestibility (DIVMS). Both varieties can progress in soils with physic limitations and retained the persistence and adaptation of the initial variety. The remaining genetic variability is still important despite a third cycle of selection applied to the original variety.

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Towards productive summer dormant cocksfoot (*Dactylisglomerata* L.) for sustainability of forage production under severe summer drought

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Poster No. 28

Abstract

In rainfed Mediterranean areas and southern Europe, the persistencyof forage crops and natural grasslands will be challenged the greater incidence of drought predicted under climate change. To maximise sustainability of forage production, perennial forage species have a number of advantages in comparison to the predominantly used annuals. They show a high water use efficiency because they can utilize water throughout the whole year, they halt erosion, restore soil fertility and enhance forage production especially after summer droughts.Mediterranean types of *Dactylisglomerata* and *Festucaarundinacea*have been shown to bevery persistent under water deficit. Moreover, a few types of these perennial grassescan be summer dormant, and therefore even more dehydration tolerant under very severe drought.This efficient strategy of summer dormancy has nevertheless been correlated with low biomass production in rainy seasons. The objective of this study was to analyse the genetic and eco-physiological determinisms of summer dormancy and biomass production in cocksfoot. For this purpose, the F1 progeny between a Mediterranean summer dormant genotype from the variety ‘Kasbah’ and a Mediterranean summer active genotype from the variety ‘Medly’ is under study. The first results show that despite an expected negative correlation (*r*=-0.35) between summer dormancy measured by the level of senescence in summer under irrigation and plant growth under favourable conditions in spring, some genotypes exhibit the same summer dormancy level as the genotype of ‘Kasbah’ and a higher biomass production than the genotype of ‘Medly’. The genomic regions implied in the variation of these traits are under study and should be useful for breeding. These results are promising for the development of new cocksfoot varieties combining both summer dormancy and high forage production.

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Combining abilities of diverse alfalfa (*Medicago sativa* L.) varieties

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Poster No. 29

Abstract

The objective of this study was to test general combining ability of the diverse alfalfa varieties, with the main goal to improve local varieties with good parental gene donors. Foreign varieties which showed good adaptation on agro ecological condition in Serbia in previous investigation were chosen as possible gene donors. Parental lines selected from domestic varieties K 22, K 23 and K 42 were crossed with 10 diverse alfalfa varieties. Progenies from crosses were sowed in nursery and observed during second and third year of utilization at the experimental field of the Institute for forage crops in Krusevac. Results showed that the significant differences between progenies. Variation among progenies for forage yield and forage yield components (plant height, number of steam per plant, etc) were primarily attributed to general combining effects. Varieties Vali and Pointer for all traits showed negative GCA, and those varieties are not suitable for improving domestic selection material. General combining ability showed that variety Magna had the best GCA with all domestic varieties for forage yield and it’s an excellent donor for improving these traits. For increasing number of steam per plants, the best GCA had varieties Integrity and again Magna. Those varieties demonstrated favorable genetic potential that could contribute to the improvement of alfalfa yield in further breeding program. The best progeny will be used as parental line in new synthetic variety.

Resistance of perennial ryegrass varieties to stem rust

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Poster No. 30

Abstract

Stem rust (*Pucciniagraminis* f. sp. *graminicola*) is very important disease in grass seed production. Since 2000, very early occurrence stem rust was observed at some locations of the Czech Republic on perennial ryegrass seed crops.Seed yield of some perennial ryegrass varieties was decreased to 10 % due to attack this rust (Cagaš *et al*., 2010). Intensive attack of stem rust apparently be connected with favourable temperatures in spring period (Lellbach, 2000), which have increasing tendency in last years.However, stem rust is disease which negatively influenced not only seed yield but also quality of seed.

To evaluate the susceptibility of selected perennial ryegrass (*Loliumperenne* L.) varieties and ecotypes were in 2010 at Grassland research Station at Zubří established field and greenhouse trials. In total, 59 varieties and 3 ecotypes were tested in both types of trial. In the greenhouse trial the artificial infection of stem rust spores were done. Rust incidence was scored according to formerly published methodics (Cagaš, 1978). In this trial, which results are significant mainly for breeding aim purposes, showed high susceptibility of most varieties and ecotypes to stem rust.Very low or zero number of plants with symptoms of infection was recorded only at cultivars“Score”, “Kokomo” and “Advent”. At other cultivars or ecotypes the number of affected plants was drawing from 75 to 100 %.Connection between findings obtained in field orgreenhouse trials was observed only at of several cultivars (e.g. “Bareuro”, “Bargold” and all ecotypes). At some case were to be results in direct antagonism (e.g. “Kertak”). Usage of greenhouse tests for assessment of field tolerance is so questionable.

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Traits stability of Italian ryegrass tetraploid cultivar during seed production in diverse environmental conditions

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Poster No. 31

Abstract

Tetraploid cultivars of Italian ryegrass (*Lolium multiflorum* Lam.) have been developed and realised in an attempt to improve forage yield and quality. But very often tetraploid cultivars are very vulnerable in production by unfavourable weather conditions. The objective of this research was to determine if the stability of Italian ryegrass tetraploid cultivar K29t seed production in very variable and contrasting environmental and meteorological conditions in Serbia. The study was conducted in four consecutive years and generations of seed multiplication. Every year seed was harvested from the primary growth in first production year after establishment. Data of investigated traits (heading and harvest dates, tiller length, spike length, number of spikelets per spike, dry matter yield, seed yield, seed germination and 1000 seed weight) were recorded, including different management practices (stand densities and nitrogen applications). Three ways ANOVA was performed in order to analyze statistical significance of differences.

It was determined that levels of seed yield, dry matter production and tiller length were most affected by environmental factors in different production years, but also in different management practice. On the contrary, spike length, number of spikelets per spike, seed germination and 1000 seed weight were less influenced by different management, in exception of extremely arid 2003 year. Variation in seed yield was more related to variation in seed number than to variation in seed weight and it was ranged from 785 kgha^{-1} in very dry year to 1559 kgha^{-1} in optimal conditions. Therefore, seed of tetraploid Italian ryegrass cv. K29t can be produced in diverse environmental conditions in Serbia without detrimental shifts in cultivar characteristics and significant reduction of seed yield

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Genetic variability of the most important traits of meadow fescue genotypes

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Poster No. 32

Abstract

Meadow fescue (*Festuca pratensis* Huds.) is important perennial grass for animal feed production on grasslands and pastures. Basic requirement for successful breeding of this species is variability of the initial material. The collection examined consisted of eleven genotypes (five breeding populations and six cultivars) of meadow fescue. Trial was conducted as spaced plant nursery with 30 plans per genotype in two years. The aim was to determine genetic variability for the most important phenological (heading) and morphological traits (plant height in the first and the second cut, sward diameter, length of leaf, width of leaf, the leaf number, terminal internodes length and the number of tillers per plant), and annual biomass yield per plant. Investigated material has demonstrated significant variability within the genotypes for all studied parameters. The highest average within population variability was determined for the number of tillers (CV 38.46-69.60 in 2006. and 43.67-87.41% in 2007.), while the lowest variability was determined for time of heading (CV 2.37-6.8 in 2006. and 8.06-23.92% in 2007.). The data were analyzed by ANOVA for each year separately. Among populations variability was high statistically significant for all investigated traits. Genetic relationship of examined meadow fescue genotypes was illustrated by cluster analysis. High level of genetic variability in this collection indicates that the breeding process can make progress in the further.

Assessment of sensitivity of different alfalfa genotypes to *Colletotrichum destructivum*

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Poster No. 33

Abstract

Southern anthracnose or crown rot, caused by *Colletotrichum destructivum*, is a diseases which has been detected on alfalfa in Serbia. During a 6 year period, especially in summer and autumn, plants with antracnose symptoms were studied in alfalfa field. Stem infection resulted in wilting and death of the upper portion of the steam, giving rise to the characteristic “shepherd's crook” symptom. To examine the level of sensitivity of different alfalfa genotypes in experimental conditions, 6 isolates: Coll-3, Coll-8; Coll-9, Coll-10, Coll-18 and Coll-75 (*C. destructivum* originating from Serbia and one reference isolate CC657 *C. destructivum*) were used. Ten commercial alfalfa cultivars of different origin (K-1, K-28, Zaječarska 83, Osječka 12, NS Slavija, Banja Luka, Affinity 401 + Z , Florida 77, Vernal S i Perry) were examined in this study. Infected plants showed typical symptoms of anthracnose. Necrotic lesions appeared on the plant stems and gentle bending of the top of the upper third of stems occurred on tested plants. Necrotic lesions were spread further onto the whole plant and in some plants led to the withering. Assessment of the damage by pathogen inoculation was based on the scale of 0-5 by methods by Ostazeski et al., 1969. The results were processed by analysis of variance as factorial experiment, where the first factor was the cultivar and the second factor were selected isolates. Investigated cultivars showed various resistances to different *C. destructivum* isolates.

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The influence of climatic conditions on the hay yield of the certain types of grass

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Poster No. 34

Abstract

The aim of this study was to examine the possibilities of cultivation and yields of perennial grasses of foreign origin in the western and eastern Croatia. Accessions within a species of grass were minimal distinguished by yield of green mass and hay, while the differences were significant for yield of crude protein. The most varied characteristic was yield of protein per unit area (coefficient variability CV 8.16%). The location impact is significant only for the yield of hay and crude protein, while the impact of the year along locations was not significant for all characteristics. Impact of year as a separate variable had a significant influence on all the properties of the yield. The highest yield of green mass and hay had Italian ryegrass (TLJ2) while cocksfoot (KO2) achieved the highest yield of protein.

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Alfalfa breeding under conditions of high soil acidity

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Poster No. 35

Abstract

Alfalfa is one of the most productive forage crops. It is especially valuable for biologization of agriculture. However, by its biological characteristics alfalfa plants normally develop only in soils with 6.0 to 7.5 pH of salt extraction that is close to neutral.

At the same time in Ukraine every fourth hectare of soil is acid, in Polissya and Forest-Steppe almost every second hectare is acid (49,7-47,4%). This state of agricultural lands necessitates the development of breeding technology on edaphic selection and breeding of alfalfa varieties that can function normally and produce in soils having high pH.

Researches on the optimization of methods of creating alfalfa breeding material with high adaptive ability to be grown in acidic soils were conducted on experimental fields of the Institute of Feed Research and Agriculture of Podillya of the NAAS (1982-2010). Soils were grey podsol with 5.0- 6.5 pH of salt extraction and hydrolytic acidity of 2,1-2,4 mg / eq. per 100 g of soil. Such a diversity of soils allowed us to allocate a selective background with 5,0-5,5 pH for further researches. Breeding varieties, local populations of wild alfalfa and yellow alfalfa, their hybrids and other breeding material were used as a source material.

The results of long-term researches show that alfalfa varieties tolerant to soil acidity and able to generate relatively high yield of green mass and seeds are of especial practical interest in our conditions. Expanding of adaptive characteristics of alfalfa to pH was performed by successive replanting of hybrids bred at selective background with 5,0-5,5 pH.

Our researches have shown that a single cycle of selection increased productivity of forage mass of some populations by 40-50%, double - 80-110%, compared with the original populations.

As a result of competitive variety testing carried out in 2001-2010, a number of promising breeding numbers of alfalfa which exceeded standard varieties Regina and Poltavchanka by 50-80% against the background of 5,0-5,5 pH have been selected. Thus, the first stage of breeding of new alfalfa variety tolerant to conditions of growing on the sites having acidic soils with 5,0-5,5 pH was completed.

Breeding number 4/95 called Synyukha, transferred to the state variety testing in 2006 and listed in the State Register of Plant Varieties of Ukraine in 2010, belongs to the hay-pasture type. Along with tolerance to soil acidity it is characterized by resistance to root rot and extended period of productive longevity (3-4 years), it forms seed yield up to 0.6-0.7 t/ha. It is recommended to be spread in Polissia, Forest-Steppe and Steppe zones of Ukraine.

Ideotypes of forage pea (*Pisum sativum*) cultivars

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Poster No. 36

Abstract

Pea (*Pisum sativum* L.) is one of the most important grain legume crops in temperate regions worldwide. In many countries of Europe, West Asia and North Africa, it is also used in feeding ruminants in the form of fresh forage, forage dry matter, forage meal, silage, haylage and straw. The goals of breeding pea for forage significantly differ from those in breeding pea for grain. A large majority of currently ongoing breeding programmes on forage pea develop cultivars completely or mostly from local or introduced populations of *P. sativum* L. subsp. *sativum* var. *arvense* (L.) Poir. Such cultivars are characterised by long stems with indeterminate growth, long internodes, great number of nodes, large stipules and three pairs of leaflets, purple flowers and low seed yields in comparison to typical feed pea cultivars. They also have prominent winter hardiness, late maturity and large forage losses due to an extremely poor lodging tolerance, being the reason why these cultivars are mostly grown intercropped with cereals. Much of said traits, such as large aboveground biomass resulting from long stems or large leaves are important segments of the ideotypes of a forage pea cultivar. More recent improvements include determinate stem growth and moderately great stem length, in order to reduce withering of at least a whole lower half of leaves on the plant, as well as a leaf proportion in the aboveground biomass higher than 0.50, improved earliness and a forage dry matter proportion of about 0.25, increasing crude protein content and decreasing both neutral and acid detergent fibre and lignin content in forage dry matter. A successful combination of all these desirable traits may result in a forage pea cultivar producing about or slightly more than 10 t ha^{-1} of forage dry matter and about 2 t ha^{-1} of forage crude protein, able to easily fit into diverse cropping systems. The latest trends in forage pea cultivars brought forth the first semi-leafless cultivars with excellent standing ability and enhanced seed production, where numerous and large stipules successfully replace the missing leaflets in providing quality forage.

The sum of effective temperatures for seed production of birdsfoot trefoil (*Lotus corniculatus* L.)

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Poster No. 37

Abstract

Genetic potential for seed yield of Birdsfoot trefoil (*Lotus corniculatus* L.) is 1200 kg/ha, and the average yield in the world as well as in our country is at a level below 200 kg/ha. Shattering of pods significantly limits the efficient production of seeds. Seed maturation of Birdsfoot trefoil is uneven, and it begins in the lower part of the plant and continue to the top when fully ripe pods break easily and the seeds drop off. There is a large number of populations of *Lotus corniculatus* L. in our agroecological conditions that possess high genetic variability. Rich source of variabilities for a number of important agronomic traits is very important in order to find the most productive and for their introduction into the further breeding process. Breeding process for Birdsfoot trefoil is quite long and demanding. The aim of this study was to combine generative and vegetative propagation methods to get the seeds and to calculate effective temperature units with the least possible loss of seeds. Harvesting was carried out when 65% of plants had dark brown pods. The total number of days from transplanting to harvest in the first year was 102, and the total sum of effective temperatures in this interval was 1647,9 °C. During second year it was noted that the first cut for forage required 95 days, or the sum of the temperatures 1582,5 °C. The required sum of effective temperatures for a period from the first mowing to the beginning of flowering of the second cut was 724 °C, up to the full flowering 940,6 °C, and for obtaining the seeds from a second cut 1669,5 °C. Based on these parameters, it can be concluded that for seed production from second cut plants need a period of 70 days. In the third year, when the plants were in bud stage, cuttings were taken for vegetative reproduction and rooting lasted 35 days. Period from collection of cuttings till the seed maturation lasted 104 days with a total sum of temperatures of 2225,5 °C. With vegetative reproduction basic material was obtained, which is prerequisition for leaning genetic purity of genotypes with positive attributes. Based on these parameters, it can be concluded that in the conditions of Banja Luka region, in one growing season, plants of Birdsfoot trefoil can be multiplied vegetatively and they can produce seeds.

The Czech core collection of *Medicago* spp.

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Poster No. 38

Abstract

Altogether 52 characters were evaluated in the set of 457 accessions (varieties, newly bred varieties and wild forms collected in the nature) of the world collection of the *Medicago sativa, Medicago x varia* and *Medicago falcata*. Thirty plants of each origin were planted on the field. Ten of them were evaluated in the years 2005 and 2006. All the evaluated characters were included into the analyses. Missing values were replaced by mean value. Cluster analysis was performed in the software Statistica for Windows for all the accessions together. Complete linkage method was used for clustering and Euclidean distance as the measure of distance.

The life-span of leaves as a determinant of crop plant productivity

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Abstract

Senescence is the last phase of development before death of a whole plant or plant organs such as leaves. The major purpose of leaf senescence is the remobilization of nutrients, in particular nitrogen, for building up new leaves or filling up seeds and fruits. Leaf senescence can, however, also reduce crop yield, when it is induced prematurely under adverse environmental conditions. Most of the leaf nitrogen is found in the photosynthetic apparatus of chloroplasts which are stepwise dismantled during leaf senescence. Besides Rubisco and other stroma proteins, the chlorophyll binding proteins of the thylakoid membranes are degraded as obvious by a decline in chlorophyll content. With the aim to enhance productivity, a number of *stay-green* cultivars showing a prolonged leaf area duration have been selected by conventional breeding. In many cases, a positive correlation between leaf area duration and biomass production has been observed. In only few cases, however, a positive correlation between leaf area duration and seed yield was observed. In several crops, the *stay-green* phenotype is observed to be associated with a higher drought resistance and a better performance under low nitrogen conditions. The European project “CropLife” focuses on processes determining leaf lifespan in cereals and grasses. Initiation and progression of leaf senescence is explored in transgenic barley lines and in ryegrass genotypes. The results of the fundamental research on leaf senescence are expected to path the way to more efficient breeding strategies aiming to prolong leaf area duration of forage and turf grasses.

Targeting reproductive traits for more efficient forage grass breeding

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Abstract

Perennial ryegrass (*Lolium perenne* L.) is one of the most important grassland species of temperate regions worldwide. Due to its allogamous nature, perennial ryegrass has– so far – been improved as open pollinated populations or synthetic varieties.

Using novel grass breeding schemes to increase our efficiency to exploit the genetically available heterosis may have the potential to improve the genetic gain for agronomically important traits such as biomass yield.However, current attempts to produce classical hybrids in forage crops have major limitations: Firstly, inbreeding is hampered by an efficientself-incompatibility (SI) system promoting cross pollination. Secondly, plant performance and fertility is affected by a severe inbreeding depression that is characteristic of allogamous species. Moreover, tools to control pollination for efficient hybrid seed production are currently not available.

Combining research activities for traits involved in plant reproduction such as the two-locus SI system, fertility restoring self-compatibility (SC), cytoplasmic male sterility (CMS) and doubled haploid (DH) induction will pave the way for a shift towards hybrid breeding in forage grass species. Starting with an overview about the latest scientific achievements for these traits in perennial ryegrass (which serves as a diploid model for other major grass species), we will evaluate the prospects of using SI, SC, CMS and DH as breeding tools for efficient forage grass improvement.

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Emerging knowledge from genome sequencing of model plants and fodder crops

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Abstract

Extensive insights into genome composition, organization, and evolution have been gained from plant genome sequencing and annotation projects. This analysis of crop genomes has provided surprising results which have important implications for plant origin and evolution, genome polyploidization, species adaptation and gene functional modulations. The work on the maize (*Zea mays* L.) genome provides a clear example that the genetic diversity among accessions is not restricted to simple DNA polymorphisms, but includes the presence/absence of large sets of genes. *Medicago truncatula* and *Lotus japonicus* were studied as model species for forage legumes (alfalfa, *Medicago sativa*; clovers, *Trifolium* sp.; pea, *Pisum sativum*; vetches, *Vicia* sp.). Knowledge acquired from these model species was used to develop tools, such as molecular markers, for the crop species; genetic maps have also proved to be highly syntenic among legume species. Mapping populations of *M. truncatula* were exploited to identify genes involved in variation for traits of agronomic interest. Nowadays, next generation sequencing offers the possibility of sequencing heterozygous species. Several projects are on-going in France and the USA on alfalfa in which the *M. truncatula* genome is used as a reference to align alfalfa sequences as in a re-sequencing strategy. In forage grasses, efforts in several European countries and Australia have focused on the sequencing of diploid perennial ryegrass (*Lolium perenne*). An inbred genotype, for which a number of resources have already been established, was chosen. These resources include a BAC-based physical map and associated BAC-end sequences, genetic maps and annotated transcriptome sequence data. Illumina, 454 GS-FLX and third-generation sequencing technologies were used to sequence a range of genomic libraries for *de novo* assembly. These combined resources are being used to assemble and annotate the perennial ryegrass genome. The large set of SNP markers produced by these projects will be useful for genetic mapping of segregating populations, association studies and selection genomics. Allogamous species in which populations have considerable variation at the trait and genome levels but also low population structures are suitable for developing association studies and also genomic selection plant breeding approaches.

Young researcher presentation

Influence of short-term drought conditions and subsequent re-watering on the physiology and proteome of *Lolium multiflorum / Festuca arundinacea* introgression forms with contrasting levels of tolerance to long-term drought

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Abstract

Drought is one of the most important environmental stresses limiting plant growth and development. *Festuca arundinacea* (tall fescue) is a model species for the research on tolerance of water deficit in forage grasses. Contrary, *Lolium multiflorum* (Italian ryegrass) has better forage quality but lower tolerance to abiotic stresses. The two species hybridize and their intergeneric hybrids and introgression forms state the unique objects to perform research on the molecular nature of tolerance to drought. The aim of the work was to compare how the *L. multiflorum / F. arundinacea* introgression forms react to long-term drought in the field and short-term drought in laboratory conditions. Partially fertile F_1 *F. arundinacea* (6x) × *L. multiflorum* (4x) hybrid was applied as an initial plant material into a backcross breeding program with tetraploid *L. multiflorum* cultivars. The plants from BC_4 generation were then used in comprehensive physiological and proteomic research, involving (*1*) the selection of genotypes with distinct levels of tolerance to long-term drought (14 weeks), (*2*) measurements of parameters describing the physiological status of the selected plants during short-term drought (11 days), (*3*) the analyses of protein accumulation profiles using two-dimensional electrophoresis, (*4*) and mass spectrometry identification of proteins which were accumulated differentially between the selected genotypes at different time points of short-term drought. Two genotypes with distinct levels of tolerance to long-term drought were selected. The genotype no. 7/6, poor with reference to yielding potential during 14 weeks of drought and to ability of re-growth after re-watering, was less affected by the stress during 11 days of drought, manifesting higher leaf water content and more intensive photosynthesis. The genotype no. 4/10, more tolerant to long-term drought, had better ability to repair its damaged cell membrane after watering following short-term drought period and was also characterized by lower transpiration rate in drought. These traits could be crucial to survive longer drought period in the field. Out of 455 proteins analyzed, 17 were shown to be differentially accumulated between the two genotypes and these were identified. Higher photosynthetic capacity of the genotype no. 7/6 was due to more efficient Calvin cycle supported by higher accumulation level of crucial proteins, involving the chloroplast aldolase, and 14-3-3 protein.

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A genetic association between leaf elongation rate and flowering time in perennial ryegrass

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Abstract

Leaf elongation rate (LER) is an important component of forage grass dry matter yield. Leaf lamina initiate rapid expansion during the winter at a time that coincides with lengthening days but this extension is not associated with any large environmental shift such as an increase in light intensity or temperature. The rate increase precedes any anatomical indication of floral initiation in apical meristems (double ridge formation) by around two to three weeks (Parsons and Robson, 1980). We measured LER in a perennial ryegrass (*Lolium perenne* L.) pair-cross mapping family that has been extensively mapped with more than 1 200 SNP markers generated by Illumina's Golden Gate® assay, and anchored to the seven linkage groups as defined by Triticeae nomenclature with seven micro-satellite markers originally developed by ViaLactia Biosciences. Measurements were made at a time when the plants' leaves were beginning to expand rapidly with leaf extension rates varying between approximately 0.4mm and 1.1mm h^{-1}. We obtained a significant correlation between LER and ear emergence (the date at which inflorescences emerge above the flag-leaf ligule) measured in two separate seasons ($R^2_{[df=83]}$= 0.17 and 0.11; P<0.001 and P<0.01.

A QTL for LER was revealed with a maximum lod score of 13.8 that coincided with a major ear emergence date QTL on linkage group 7 which is determined by the action of *Arabidopsis* flowering initiation gene homologues, Flowering Time Locus (FT) and Constans (CO) (Armstead et al. 2008). This is the first evidence that increase in LER is triggered by a critical long day photoperiod, probably mediated by the same transcription factor (Constans) expressed in leaves, in advance to the trigger for the floral initiation response on translocation of the FT product to apical meristems.

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A cost effective approach to generate a large number of markers for genotyping in perennial ryegrass suitable for whole genome association studies

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Abstract

High throughput next generation sequencing approaches have had a radical impact on the ability to genotype populations of plants for a variety of purposes, enabling the generation of far higher marker densities than previous methods. One such technique, referred to as Genotyping by Sequencing (GBS) is rapidly becoming the method of choice for genotyping applications in plant species. We have created a genetic linkage map of perennial ryegrass F2 population based on a GBS approach. This F2 population has been used before to map segregation distortion, biomass accumulation, crown rust and global metabolites. For this population a high density genetic linkage map consisting of a mixture of SSR and DArT had been generated previously and served in this study to asign markers to linkage groups. The parents and a subset of 186 individuals of the original population were used to generate reduced representation libraries (RRLs) with two separate methylation sensitive enzymes, *ApeK1* and *Pst*. These libraries were barcoded, to allow identification of individual samples, pooled, and subjected to single end sequencing on the Illumina HiSeq2000 platform, producing approximately 300 million reads for each of the two libraries. We describe the pipeline we are using to identify SNP variation and other polymorphisms from these data (via alignment to a genome assembly based on mid-level Illumina-based shotgun sequencing of one of the inbred line parents), and to convert them into segregation datasets suitable for genetic linkage mapping. We present a genetic linkage map comprising several thousand loci, and explore challenges such as heterozygote detection and creating high density genetic linkage maps using GBS data, in which cumulative low genotyping error rates cause map expansion when current standard mapping approaches are applied.

Identification of QTLs for leaf senescence in perennial ryegrass (*Lolium perenne L.*)

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Poster No. 39

Abstract

Perennial ryegrass (*Lolium perenne* L.) is a popular forage grass due to high digestibility, palatability, good tolerance to grazing and adequate seed production. Perennial ryegrass is a diploid species with seven pairs of chromosomes (2n=2x=14) and a relatively large genome size of about 1660 Mbp. It is a wild undomesticated species with inherent outcrossing characteristics thus subject to natural selection in highly diverse environments over many generations. Its genetic diversity is an excellent source of trait variation. It is a member of the Triticeae tribe of grasses and has high levels of synteny with major crops species such as barley and wheat. In perennial ryegrass, lifespan is important as a major determinant of productivity. Identification of genes that can prolong photosynthesis and delay senescence processes will be vital not just for understanding of the mechanism of senescence but also for practical purposes such as improvement of productivity, silage and hay quality and stress tolerance. An F2 *Lolium perenne* population with a total of 188 plants was used for the characterization of leaf senescence parameters. Four different phenotyping activities were conducted namely: (1) detached leaf senescence under natural light senescence; (2) dark-induced senescence of detached leaves; (3) attached leaves grown in controlled environment and (4) attached leaves grown under green house condition. Chlorophyll content, color index and chlorophyll fluorescence were recorded every three days for twelve days for the detached leaf experiments The same senescence parameters were recorded every ten days for attached leaf senescence phenotyping. Several QTLs for detached leaf senescence were identified on the chromosomes 1, 2, 3 and 6. Further work will include a more detailed phenotypic characterization and identification of genes underlying the QTLs. Selection of plants based on the genotypes of the molecular markers flanking the QTL regions identified will be made to produce populations for performance testing under controlled environment and field situations.

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Expression of genes encoding key transcription factors during senescence in ryegrass

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Poster No. 40

Abstract

Senescence is the last stage of leaves development. It is very complex process of controlled plant death involving the relocation of nutrient from leafs to seeds.Due to thecomplexity and interweaving with biotic and abiotic responses the molecular mechanisms underling senescence remain to be elucidated.

My research is part of the Europeantraining networkCropLife. It is constructed of PhD students and Postdocs all over Europe which are making research on senescence. One of network aims is implementation fundamental knowledge of the senescence regulatory and molecular mechanism into breeding program.

Senescence is closely connected with plant yield. In many cases the positive correlation between delayed senescence and plant yield has been observed.Goal of my research is to find novel senescence markers which can be used for ryegrass selection.Genes encoding senescence transcription factors already known in barley represent candidates in ryegrass due to the genome syntenies. One of the putative markers is *S40*.

Gene expression analysis will be performed with *L. temulentum* as a model for perennial ryegrass.Gene expression during senescence will be analyzed by RNA seq. Also, different ecotypes of *L. temulentum* will be used for phenotyping and gene expression analysis during senescence. Long term idea is to find suitable parents for F1- cross with preferred characteristics which will then be transferred to *L. multiflorum* and *L. perenne* via backcrossing with the F1-cross from *L. temulentum*.

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Heterosis in alfalfa breeding

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Poster No. 41

Abstract

Alfalfa is highly productive forage species, with great potential for hay production. Breeding alfalfa for yield has not been very successful compared to other species.The progress of alfalfa breeding has been slow, most notably due to its complex genetic structure (autetraploidy), and tetrasomic inheritance present in alfalfa. Alfalfa breeding programs are based on recurrent phenotypic selection with or without progeny testing, to accumulate desirable alleles at high frequency into a population (Li and Brummer, 2012).Previous researchclearly shows existence of heterosis in alfalfa (Milić et al., 2011; Li and Brummer, 2012). The genetic control of major agronomic traits is determined by both additive gene action (accumulation of frequency of desirable alleles represented by significant GCA effects) and nonadditive gene action (complementary gene interactions represented by significant SCA effects). This type of gene action expression in alfalfa also determines the way in which breeding is carried out and brings about changes in the methods used.It has also given rise to the idea of the semi-hybridbreeding of this crop which involves: breeding alfalfas within the population, identification of heterotic germplasm, and the production of seed of the population hybrid. In producing the seed of population hybrids, traits that are not directly related to hybrid vigor can be important as well. For instance, the maturation periods of the heterotic groups should be of equal length so that their ripening is simultaneous in order to secure as much cross pollination as possible (Milić et al., 2011).In order to get as much crossing as possible in alfalfa, the reproductive biology of this crop should be studied in greater detail for the purposes of hybrid seed production, no matter how unattainable this goal might seem at the present level of knowledge.Used germplasm should be genetically divergent, but its potential maintenance can be carried out more easily and more safely in geographically separate centers, which is why genetic divergence often coincides with different geographic origin.The studies of the relationship between the molecular variability of alfalfa populations and heterosis in their hybrids could contribute to a more precise selection of parental populations to be used for crossing in semi-hybrid alfalfa breeding procedure,with aim to reduce number of necessary crossings and therefore make future alfalfa breeding programs more efficient. (Nagl et al., 2011). Future tasks of alfalfa breeders should be to discover how to translate heterosis from single plants in hybrids planted in dense stand to generate “yield for free“(capture heterosis) in alfalfa semi hybrids.

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Inheritance of crown rust resistance in a perennial ryegrass genotype of the cultivar Arvella

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Poster No. 42

Abstract

Crown rust, caused by *Pucciniacoronata* f. sp. *lolii* is a common disease of Italian (*Loliummultiflorum*) and perennial ryegrass (*L. perenne*) in Europe. To study the inheritance of resistance to this disease, a resistant and a susceptible genotype of the cultivar Arvella wereintercrossed. A resistant progeny was backcrossed with a susceptible genotype from the cultivar Aurora to generate an F_2 population. Out of this population, two parents were selected on the basis of crown rust reaction. Each of the two resistant parents was crossed with a susceptible Aurora plant to generate two sets of F_3. One resistant F_3 plant from each set was used to generatean F_4 populationby crossing with a susceptible Aurora plant.

All parents, the F_2, two F_3 and two F_4 populations were screened against three single pustule derived isolates of crown rust in a detached leaf segment test under growth chamber conditions.

The first set of F_3 and the corresponding F_4 population fitted a segregation ratio of 1R : 1S when inoculated with the isolates K and F. Isolate A was virulent to all the progeny tested. In contrast, in the second set of F_3 and F_4a segregation ratio of 1R : 1S was observed for the isolates K and A. Isolate F was virulent to all the progeny tested.

The disease reaction of the F_2 and the segregation patterns of the F_3 and F_4 generations showed that resistance to crown rust in the Arvella genotype is controlled by at least two independent single dominant genes.

Variation of cell wall digestibility in fodder grasses and a perennial ryegrass breeding pool

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Poster No. 43

Abstract

Improved cell wall digestibility (DNDF: digestibility of neutral detergent fibre) of fodder grasses may contribute to a higher dry matter intake and a better grass protein use efficiency by the ruminant. We determined DNDF of several fodder grass species (perennial and Italian ryegrass, meadow and tall fescue, cocksfoot and timothy) and families of a perennial ryegrass breeding pool by NIRS (near infra red spectroscopy). The calibration of the NIRS is based on in vitro digestibility and NDF analysis (Van Soest et al., 1991).

Among the grass species perennial ryegrass had the highest and timothy the lowest mean annual cell wall digestibility. Tetraploid perennial ryegrass varieties showed a higher DNDF than diploids.

The variation of the DNDF among families of perennial ryegrass is described. We calculated the correlations between the DNDF of the single cuts and the DNDF on an annual basis. Also correlations between DNDF and dry matter yield and content, organic matter digestibility, content of water soluble carbohydrates and protein content are shown. Selection for higher digestibility by increasing sugar content or cell wall digestibility is discussed.

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Young researcher poster

Transcriptome profiling under hardening/de-hardening conditions in *Lolium perenne* by RNA-sequencing

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Poster No. 44

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Abstract

The predicted future unstable winter climates with multiple thawing/freezing will pose new types of winter stresses. Cold hardening effectively increases freezing tolerance in many temperate plant species. Understanding the mechanism that regulates cold hardening is important for cultivar development of perennial ryegrass which lacks adequate winter survival. In this research our aim was to study global changes in the transcriptome as affected by vernalization and cold acclimation in genotypes with different vernalization requirements. Clones of two genotypes from the Norwegian *L. perenne cv* Fagerlin which differed in vernalization requirement under natural field conditions, i.e. genotype 201 which flowered without vernalization and genotype 204 which needed vernalization to flower, were given two pretreatments: 1) Vernalization for 10 weeks at +6 ºC and 2) No vernalization, i.e. grown at +15 ºC. Following this the plants were hardening for 4 weeks at +2 ºC, then de-hardening for 8 days at +9 ºC followed by re-hardening for 2 weeks at +2 ºC. Freezing tests were conducted after every treatment with test temperatures ranging from -4 ºC to -20 ºC. Crown samples (3 biological replicates) were collected at each time point and used for RNA extraction. LT_{50} results showed that vernalization (pre-hardening) at +6 ºC led to higher frost tolerance than pre-hardening at +15 ºC. Genotype 204 (with vernalization requirement) pre-hardened at +6 ºC showed the largest capacity to re-harden (3-6 ºC) thus showing a significant difference between the two genotypes. An RNA-Seq approach was taken to analyze the transcriptome of the crown tissue. RNA was extracted from a total of 54 samples for cDNA library preparation and paired end sequencing was performed using Illumina Hiseq 2000. Global transcriptome differences between the two genotypes as affected by vernalization, hardening and de-hardening will be presented.

Allele frequency of local maize inbred lines

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Poster No. 45

Abstract

The objective of this study was to screen genetic divergences of 32 inbred lines with different protein content, in F4 generation of self-fertilization, origin from domestic populations of Eastern Serbia.

In order to speed up the selection process, it is important to select the genotypes with desirable properties in early phases. Isoenzymes as a direct product of the genes can be used as genetic markers in the selection process. Different number and distribution of certain alleles in inbred lines of maize indicates their mutual distance and genetic divergence. Differences of the investigated inbred lines can be showed by the presence or absence of specific alleles in loci and their frequency.

The highest number of alleles (14) was found in line number 5, many as 14 inbred lines had 13 alleles, while the one line had 12 alleles (line No. 9). The three lines each had 10 and 11 alleles, while one line had 9 alleles (line No. 30). Only eight lines were homozygous for all loci (3, 7, 10, 21, 24, 26, 28 and 30).

According allele frequencies were allocated to three groups of lines (A, B and C), with different number. The first and second groups are joined together at a distance 4.2 and they form a new group of lines, which is connected to the third group of the most distance 7.

The third group of lines is clearly separated from the first two groups. Lines 26, 28 and 32 can be viewed as independent genotypes, which join to the lines of the third group at a distance 2.25.

These results allow a clearer evaluation of the lines during the process of selection and (together with other desirable features) faster choice of material for further selection.

Breeding of rye grass varieties adapted to biotic and abiotic stress by integration of innovative genomic and metabolomic tools

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Poster No. 46

Abstract

Novel breeding tools, such as genomic and biophysical technologies, may enhance the development of ryegrass varieties with improved adaptability to biotic and abiotic stress caused by man-made climate changes. Molecular markers and NGS technologies enable the accumulation of resistance genes against stem rust, crown rust and bacterial-wilt (pyramidization or gene stacking) as well as efficient back-crossing into elite germplasm. State-of-the-art functional genomic and metabolic methods also allow the detailed characterization of disease resistance mechanisms and drought stress processes at the plant organ and cellular level opening up new perspectives in the application of biomarkers in breeding for biotic and abiotic stress tolerance.

Based on results from prior projects, resistance biotests and DNA-marker analysis, one source of stem rust- and bacterial-wilt-, two sources of crown rust resistance and three essential resistance genes linked markers were selected. By the pyramidization the resistance genes are cumulated in a short-lived, wild and autogamous *Lolium temulentum* and will be transferred later into elite material through marker assisted back-crossing. The first hybrids between *L. temulentum* and *L. perenne* were obtained through embryo-rescue.

114 genotypes of *L. perenne* were tested against stem rust with isolates from southern- and northern Germany and from Oregon (USA). 51% of the material was susceptible to stem rust and the rest was moderately resistant. One genotype showed resistance against all the tested isolates and also against crown rust.

Massive Analysis of cDNA Ends (MACE) was performed with susceptible and resistant bulks (resistant vs. susceptible inoculated vs. not inoculated) of leaf-segments at 4 different testing times. Out of 120, 000 Tags could 10,000 genes be differentially expressed.

Reduced Complexity Genomic Sequencing (RCGS) was performed on 9 *Lolium* genotypes. Genotypes with possibly different genetic background showed very few identic sequence features. Nuclear magnetic resonance spectroscopy (NMR) for the extraction and measurement of samples was developed and optimized. The developed methodology allows optimum yields of information and effective processing of numerous samples simultaneously. Metabolic profiles showed clear differences between resistant and susceptible genotypes. Regarding drought stress, two experiments in a climate chamber with *L. perenne* and *L. multiflorum* showed a clear reduction in biomass in stressed plants compared to control plants. Signatures in the NMR spectra reflected the diverse reaction of genotypes to drought stress.

The project consortium consists of 5 partners: Saatzucht Steinach GmbH & Co KG, Julius Kühn-Institut, Numares Group, GenXPro and Universität Rostock.

Next generation genotyping strategies to enable genomic selection.

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Poster No. 47

Abstract

There has been limited success to date in directly applying molecular markers in traditional breeding programs. A major factor in this was the paucity of molecular markers to adequately cover the genome. While finding marker-trait associations within family populations has been possible with limited markers, the cost and time to apply such markers has meant they have only been used on a limited number of experimental crosses, and thus any identified markers may be of little use in breeding material. Finding more useful marker-trait associations in populations (e.g. breeding material) is only possible with dense marker coverage to overcome the low linkage disequilibrium present in outbreeding species. However, the rapid development of sequencing technologies means marker density is no longer a bottleneck. These advances are now enabling plant breeders to consider genomic selection as a tool to increase genetic gain in breeding programs, in a manner similar to that seen in animal breeding. We are involved in a large project (ForageSelect) that is tasked with implementing genomic selection in a traditional breeding program. The genomic selection models will be developed on a training population consisting of 1000 perennial ryegrass families. Initial work focused on selecting an appropriate strategy for genotyping of 1000 families, and the results of pilot experiments favored a genotype by sequencing approach using a frequent cutting enzyme for genome complexity reduction. This has now been applied to the genotyping of the complete training population and the results will be discussed in terms of (1) number of molecular markers discovered at different minor allele frequency thresholds, (2) coverage at each maker across populations and the level of missing genotype data, and (3) the frequency of marker discovery in protein coding sequences.

Lucerne genetic variation for response to tissue culture and *in vitro* shoot regeneration, and its relationship with other traits

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Poster No. 48

Abstract

Identifying new lucerne genotypes which respond well to *in vitro* shoot regeneration and are agronomically valuable has crucial importance for broadening the genetic base and raising the genetic value of transgenic varieties and of germplasm issued by *in vitro* selection applied to calli after mutant induction, as well as for widening the genetic background of material in functional genomics studies. Earlier work indicated that *in vitro* shoot regeneration via somatic embryogenesis or direct organogenesis is rare and subjected to complex genetic control in lucerne. The aims of this study was assessing the occurrence of this feature and its relationship with forage yielding ability and response to clonal propagation in a collection of elite genotypes.

Some 53 genotypes issued from stratified mass selection for forage matter applied to 4500 genotypes (sorted out from several Italian landraces and varieties) were evaluated for: (i) callus fresh weight and frequency of *in vitro* shoot regeneration, (ii) breeding value as two-year dry matter yield of their half-sib progenies, and (iii) percent of cloning success across three experiments. For each genotype, leaflets from different apical leaves were cultured *in vitro* on a callus induction medium in a growth chamber, assessing the calli regeneration in a culture medium lacking growth regulators after three weeks. The experiment was repeated twice.

All genotypes and, on average, over 78% of the leaflets were able to produce callus. Genotype differences in callus fresh weight per explants were highly significant and largely heritable in the broad sense ($h^2 > 0.84$) in each experiment, largely consistent across experiments (genetic correlation $r_g = 0.67$), and unrelated to breeding value for forage yield ($r = -0.01$) or percent of cloning success ($r = 0.02$) of the genotypes. Only one genotype out of 53 could produce somatic embryos and complete the cycle of *in vitro* shoot regeneration. Only 10% of its calli actually produced somatic embryos, suggesting that not all cells in the growing calli are competent for somatic embryogenesis. The efficiency of embryo conversion into rooted shoots of this genotype was 40%. Interestingly, this genotype was also top-ranking for breeding value.

Characterization of polymorphic SSR loci isolated from *Lolium Perenne* L. for genetic diversity study of *Lolium Arundinaceum* Schreb.

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Poster No. 49

Abstract

Tall fescue (*Lolium arundinaceum* or *Festuca arundinacea* Schreb.) is a cool-season forage grass that is widely grown throughout the temperate regions of the world. It is the most important perennial forage species in the northeast of Tunisia. The objective of this study was to assess the genetic structure within and diversity among populations of Tunisian tall fescue. Four spontaneous populations sampled from different eco-geographical origins were exanimate using simple sequence repeat (SSR) markers isolated from perennial ryegrass. The derived genetic distance values suggested a high level of genetic diversity in the Tunisian germplasm. The structure of populations has grouped into two clusters consisting of (i) two populations, one from Nabeul, and the other from Bizerte; (ii) the remainder originated from Jendouba. Clustering indicated possible gene flow between Bou Salem and Hammam Bourguiba populations (governorate = Jendouba). In addition, the results have permitted to precise the correlation of molecular markers with agronomic characteristics and the identification of selected genotypes. Taking advantages of the designed procedures, this study portrays the opportunity of such methods to precise the genetic diversity organisation of tall fescue. Results are discussed in relation with a sustainable characterization of diversity to provide efficient markers suitable in order to establish breeding programs.

Developing SNP Assays for LoliumPerenne: a Pilot Study for *LpCCR1*

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Poster No. 50

Abstract

Perennial ryegrass (*Lolium perenne*) is used extensively as proprietary forage in dairy farming, because of its high yield and high protein content. In order to increase the energy released from perennial ryegrass during digestion, we aim to improve the digestibility of the cell wall carbohydrate fraction. This can be achieved by reducing the lignin content. As we follow a candidate gene association mapping approach, we are currently studying the genetic diversity of genes involved in the lignin biosynthesis pathway. Tu et al. (2010) showed that down regulation of *Lp*CCR1, one of the genes coding for the enzyme cinnamoyl-coAreductase, leads to a significant reduction in lignin content. Therefore, *LpCCR1* was selected as a pilot candidate gene for developing SNP assays.

Through phylogenetic analysis, five *bona fide* CCR genes in *L.* perenne were identified in the available RNA-Seqdatabase containing transcript sequences of 14 genotypes (Ruttink et al., 2012). For SNP identification, *LpCCR1* was Sanger sequenced for an additional 6 genotypes with diverging cell wall digestibility. This way, 103 trustworthy SNPs were identifiedin the *Lp*CCR1 transcript, resulting in an overall SNP density of 7.5 SNPs per 100 nucleotides.

KASP assays were used for genotyping a selection of 12 SNPs in the AM population. 92% of these assays were validated, indicating that they are efficient in genotyping a SNP dense species. As unknown SNPs within the primer binding sites might decrease efficiency, this also shows that the genetic diversity within the 20 genotypes is sufficient for capturing most SNPs of *LpCCR1*.

As common SNPs are required for a powerful association mapping, a minor allele frequency (MAF) threshold of 10% was set. Over a genome distance of 5000 bp, thesecommon SNPs were found to be in stronglinkage disequilibrium within a non-stratified subpopulation. We conclude that the best approach for genotyping other candidate genes is to select common SNPs at the ends of the transcript and check for LD decay. If LD is strong, a limited set of common SNPs is sufficient for AM purposes.

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Genetic diversity estimation of Tunisian perennial ryegrass germplasm using different molecular methods

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Poster No. 51

Abstract

Germplasm collection as a source of genetic diversity must be evaluated for an effective use. A comparison of different methods in estimating genetic diversity is important to evaluate their utility as a tool in germplasm conservation and plant breeding. Inter simple sequence repeats (ISSR), microsatellites (SSRs) and morpho-agronomical traits markers were used to evaluate 4 spontaneous and one introduced perennial ryegrass (*Lolium perenne*) germplasm for genetic diversity assessment and discrimination power. The mean polymorphism information content (PIC) values were 0.65 (ISSR) and 0.46 (SSRs). The average pairwise genetic distance estimates were 0.57 (morpho-agronomical traits), 0.62 (ISSR) and 0.60 (SSRs) markers data sets. The Shannon diversity index was higher for morpho-agronomical traits (0.76) compared to ISSR (0.45) and SSR (0.53). The correlation coefficients obtained by the Mantel matrix correspondence test, which was used to compare the cophenetic matrices for the different markers, showed that estimated values of genetic relationship given for ISSR and SSR markers, as well as for morpho-agronomical and SSR markers were significantly related ($p<0.001$). However, morpho-agronomical and ISSR data showed non-significant correlation ($p>0.05$). Data sets from ISSR, SSR and morpho-agronomical markers allowed all accessions to be uniquely identified; two accessions could not be distinguished by the morpho-agronomical data. This study shows that ISSR and SSR markers proved to be efficient tools in assessing the genetic variability among ryegrass genotypes. The patterns of variation appeared to be consistent for the three marker systems, and they can be used for selecting valuable materials for new breeding programs, conservation of germplasm and management of Tunisian perennial reygrass genetic resources.

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AFLP analysis of genetic diversity in an association mapping panel of *Lolium perenne* L.

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Poster No. 52

Abstract

Perennial ryegrass (*Lolium perenne* L.) is one of the most important grass species in temperate regions. Genetic variation in plant architecture traits is of practical importance in perennial ryegrass breeding because of the potential of these traits invegetative production enhancement as well as the association with seed yield. However, plant architecture control in perennial grasses is less understood despite high levels of variation and versatile application of these plants as fodder or in amenity grasslands. Association analysis is a powerful tool used to identify genes responsible for variation of complex traits. Knowledge on genetic diversity of the population used in the experiment is a prerequisite for association analyses.Amplified fragment length polymorphism (AFLP) was used to measure genetic diversity in a panel of perennial ryegrass genotypes. The panel consisted of 90 cultivars, 39 ecotypes of Lithuanian origin and 64 ecotypes of Ukrainian origin. One genotype per each cultivar/ ecotype was used in a study. The three primer combinations used in the AFLP analyses amplified 227 fragments within the size range of 50–350bp. Ecotypes formed separate clusters in a principal coordinate analysis (PCA) according to their origin, while cultivars did not show any clustering pattern. Genotypes were separated into three groups (cultivars, Lithuanian ecotypes and Ukrainian ecotypes) to perform analysis of genetic variation within and between these groups. As expected, most of the variation (96.7 %) was detected within groups. Nei's gene diversity (h) and Shannon's information (I) indices were very similar (ranging from 0.2047 to 0.2094, and from 0.3247 to 0.3329, respectively), indicating similar amount of genetic diversity in all groups. The greatest genetic difference according to population pairwise F_{ST} and Nei's genetic distance (D_N) indices was detected between Lithuanian and Ukrainian ecotype groups. Nevertheless, both genetic distance indices had rather small values (F_{ST} 0.019 – 0.059; D_N0.0056 – 0.0147). In general, high genetic diversity and low differentiation between groups indicate that acquired panel of perennial ryegrass genotypes is suitable for further association studies.

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Differential expression of candidate genes during freezing test in perennial ryegrass

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Poster No. 53

Abstract

Abiotic stress not only limits the geographic distribution of forage crop production but also adversely affects it's development and yield. Therefore, breeding of plants with improved tolerance to abiotic stress becomes an important task. The objective of the current project is to develop and validate functional markers for drought and cold tolerance genes in perennial ryegrass. Differential expression study was performed for seven candidate genes putatively involved in cold (*LpIRI*, *LOS2*), drought (*DREB2A*, *Ti_Con_099*, *Ti_Con_113*) or multiple stress (*SnRK2.4*, *MSI1*) responses. The two parental genotypes, Veyo and Falster, of the perennial ryegrass VrnA mapping population (Jensen et al. 2005) were evaluated for gene expression levels in root and leaf along with stem tissues during cold acclimation for 16 days at 5 °C. After hardening the plants were frozen for 30, 60, 90 and 150 min at -10 °C and sampled for further gene expression analyses. The transcription level of *SnRK2.4* and *MSI1* in roots was twice as high in the Falster genotype compared to the Veyo genotype and reached the maximum expression level during the hardening period. The same tendencies could be seen for drought and cold response genes while the transcription of candidate genes in leaf and stem tissues varied both between hardening and freezing periods as well as between genotypes. The most divergent expression was observed for the cold response genes *LpIRI* and *LOS2*. The transcription of *LpIRI* peaked at freezing temperatures and was three fold higher in the Veyo genotype compared to Falster. The transcript level of *LOS2* reached the maximum at the hardening period, and was highly expressed in the Falster genotype while the expression in the Veyo genotype was very low. The drought response genes were higher transcribed in the Falster genotype at the hardening period. The candidate gene *SnRK2.4* showed higher expression in the Veyo at both hardening and freezing temperatures, while the transcription of the *MSI1* gene was highest in the Falster genotype at freezing temperatures. The most promising genes will be used for identification of SNPs in a perennial ryegrass association panel, and will be mapped and integrated into the VrnA genetic linkage map.

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Evaluation of two transgenes for aluminum tolerance in alfalfa

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Poster No. 54

Abstract

Acid-soil syndrome inhibits root growth and development of many plant species due to Al^{+3} and H^+ toxicity and essential nutrients deficiencies (Kochian et al., 2004). Alfalfa, *Medicago sativa* L., production is dramatically reduced in acidic, Al-rich soil. Transgenic plants of several species over-expressing organic acid synthesis and/or organic acid transporter genes have shown enhanced tolerance to aluminum (Al) (Barone et al., 2008; Delhaize et al., 2004). The objective of this research was to evaluate the effect of the citrate synthase (CS) and the plasma membrane H^+-ATPase (DcPA1) transgenes on Al tolerance in alfalfa when evaluated in a greenhouse soil assay. Transgenic alfalfa plants containing a single insertion of either CS or DcPA1 were crossed to the Al-sensitive genotypes 95-608 and 60T180-14, respectively, and a highly expressing transgenic progeny genotype from each cross was used to generate a full-sib T_2 population including four isogenic groups. Stem cuttings from 48 T_2 genotypes, consisting of 13 with neither transgene, 11 with CS only, 14 with DcPA1 only, and 10 with both transgenes, together with 8 non-transformed check genotypes were evaluated for Al/acid soil tolerance in a greenhouse assay in limed (L) and unlimed (UL) soil. Al/acid soil tolerance was assessed by measuring the ratios of root and shoot dry weight in UL soil compared to L soil. The three transgenic populations, CS, DcPA1, and CS+DcPA1, all showed higher Al/acid soil tolerance (with ratios of 0.91 to 0.98) and lower levels of Al in shoot tissue than the nontransgenic isogenic population or the nontransgenic parental genotypes, suggesting that the Al-exclusion mechanism could be driving Al/acid soil tolerance in this study. We observed no advantage of combining both transgenes in the same genetic background. These transgenes offer an efficient method to achieve enhanced Al/acid soil tolerant alfalfa cultivars, but more information is needed on their stability across generations and genetic backgrounds and their performance under field conditions.

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QTLs for nitrogen use efficiency in perennial ryegrass *Lolium perenne*

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Poster No. 55

Abstract

Nitrogen use efficiency (NUE) from soil to plant is an important trait to consider with respect to grassland improvement since it impacts on fertiliser use and nitrogen pollution of waterways. Perennial ryegrass (*Lolium perenne* L) is the most important grass species of temperate grasslands and QTL for NUE, both uptake and utilisation of N, have been characterised in a series of experiments conducted in flowing solution culture providing precise control of N form and concentration. Acquisition and utilisation efficiencies of N were characterised under growth-limiting and non-limiting concentrations of nitrate or ammonium. Significant QTL for uptake and utilisation efficiencies, and for their component traits, were observed in all cases. The presentation will examine the QTLs detected and how they were affected by the source and the concentration of nitrogen supplied. The presentation will also include the ways identified QTLs are bred into elite ryegrass genotypes using conventional and marker assisted breeding approaches.

Gene expression analysis in *Festulolium* hybrids using RNAseq

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Poster No. 56

Abstract

The advent of Next Generation Sequencing permitted sequencingwhole genomes and transcriptomeswith a depth and resolutionnever achieved before. One of the exciting opportunities has beenthe analysis of gene expression in wide hybrids. The hybridization brings homo(eo)logous genes of parental genomes under one roof and these pairs of genes mayevolve according to different scenarios during the first generations after hybrid creation. First, one gene copy may become nonfunctional by genetic and/or epigenetic changes (non-functionalization). The other option is that one copy may acquire a novel, usually beneficial function and will be preserved by natural selection with the other copy retaining the original function (neo-functionalization). The last scenario for duplicated genes is that both copies become partially compromised by mutation accumulation to the point where their total capacity has been reduced to the level of the single-copy ancestral gene (sub-functionalization).

The aim of our project is to study processes described above in initial generations (F1-F3) of hybrids of ryegrasses(*Lolium*) and fescues(*Festuca*) – Festuloliums. Weset outto develop SNP platform based onRNAseq datato reconstruct genomic composition of Festulolium hybridsand to analyze theexpressionofparental genes in hybrid progenies. We first performed IlluminaRNAseq onsix*F. pratensis* andsix *L. multiflorum* parental genotypes and mapped sequence reads from each genotype separately onto a common transcriptome reference sequence of 19,345*L. perenne*genes. This led to identification of600,000 to 900,000 putative SNPs per genotype. In each pair-wise *F. pratensis* x *L. multiflorum* combination, we selected SNPs that were homozygous within each genotype, and polymorphic between species. This yielded 30,000 to 90,000 species-specific SNPs, depending on the sequence depth and particular pair-wise combination of *F. pratensis*× *L. multiflorum* genotypes. We found that the SNPs were localized in 6,000 to 9,000 genesdistributed more or less evenly across the genome. The next step is to analyze RNAseq data from reciprocal F1, F2 and F3 hybrids,to quantify gene expression from the respective parental genomes and to assessthe putative loss and silencing of parental alleles in hybrid progenies. This work was supported by grant awards P501/11/0504 and CZ.1.05/2.1.00/01.0007.

Genetic variation and relationships of tetraploid alfalfa paternal populations and their progenies based on RAPD markers

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Poster No. 57

Abstract

The aim of this investigation was to gain information about germplasm diversity and relationships among breeding material used in alfalfa (*Medicago sativa* L.) semi-hybrid breeding program at Institute of Field and Vegetable Crops, Novi Sad (Milic et al., 2011). Having that in mind, RAPD analysis was performed on five alfalfa (*Medicago sativa* L.) parental populations and their progenies: 20 F_1 populations deriving from the reciprocal diallel crosses and 5 S_1 populations from self-pollination. Twenty selected primers generated 217 bands (loci), ranging in size from 300 to 6000 bp, with average number of bands per primer of 10.85 and polymorphism information content (PIC) value of 0.246. Percentage of polymorphic loci, effective number of alleles, expected heterozygosity and Shannon's information index were used to estimate genetic variation among tested varieties and their progenies. As expected, higher diversity was observed in progeny populations coming from cross-pollination, while progeny populations deriving from parental self-pollination maintained similar level of genetic variation to that observed in the parental populations. The gene flow among the tested populations was estimated to be 2.407 individuals per generation indicating that there is high gene flow among them. UPGMA dendogram was drawn to visualize relationships among parental populations and their progenies. It showed that F1 progenies are closer related to parental populations than S1 progenies which formed separate subclusters. It could be because selfing of alfalfa decreased level of heterozygosity that leads to more similarity inner population, but also increases the difference between parental populations and their S1 progenies (Rotili et al., 1999). Most of crosses with variety RSI 20 were grouped in the same subcluster, which is in agreement with previously observed high level of homogenity in that variety (Nagl et al 2011). Our results confirm that molecular markers are effective in assessing the homogeneity of selected self-pollinated alfalfa populations.

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Use of DArTFest Array in Breeding of xFestulolium

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Poster No. 58

Abstract

The DArTFest is DNA array consisting of almost eight thousand probes developed specifically for *Festuca-Lolium* complex. The array was proved to be an elegant tool, which enables genetic and physical mapping, marker assisted selection and the analysis on genetic diversity and genomic constitution of hybrid cultivars (xFestulolium) using species-specific markers. In this study, DArTFest array was used for testing nine Festulolium hybrids – finished variety candidates before application for official trials and standard varieties of tall fescue, meadow fescue, Italian and perennial ryegrass. Among Festuloliums, eight were of loloid types and one represented festucoid form. We analysed 7 individual plants and two bulked samples (containing 10 and 20 plants) of each Festulolium, whereas varieties of parental species were represented by bulked samples only (10 plants each). The genotyping provided an insight into genetic variability and relatedness of various Festuloliums to parental species and to each other. Among our samples, festucoid form showed lowest genetic variability and close relationship to *F. arundinacea* accessions. On the other hand, all loloids displayed higher variability compare to festucoid and clustered more or less close to ryegrass accessions. As mentioned above, DArTFest array can be used for discrimination of genomic composition in hybrid plants. We identified 773 species-specific markers, which provided clear discrimination between both parental species. The significant excess of Festuca-specific markers over Lolium-specific ones corresponds well with the origin, morphology and agronomical characteristics of festucoid form. On the other hand, results on loloid forms reflect the prevalence of *Lolium* genome over that of *Festuca*. We did not find any significant difference between analysis of individual plants and bulked samples.

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic and the European Regional Development Fund (Operational Programme Research and Development for Innovations No. CZ.1.05/2.1.00/01.0007) and by the Ministry of Agriculture of the Czech Republic (grant award NAZV QH71267).

High density array for SNP genotyping and mapping in tetraploid alfalfa

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Poster No. 59

Abstract

The development of an Illumina iSelect Infinium array that includes approximately 10,000 SNPs identified from sequencing 27 diverse alfalfa (*Medicago sativa* L.) genotypes (Li et al., 2012) can be used to accelerate genome-wide association, mapping and molecular breeding efforts in alfalfa. Individuals from a mapping population obtained from a cross between Altet-4 and NECS-141 segregating for aluminum tolerance (Khu et al., 2012), fall regrowth, lignin content and other agronomic characteristics were genotyped using the developed array. The dosage status (nulliplex, simplex, duplex, triplex, or quadruplex) of an individual was successfully distinguished using GenomeStudio software (Illumina, 2011) for most of the SNPs evaluated. A total of 3,701 SNPs were polymorphic and segregating in the progeny of the mapping population. The number of SNP with segregation ratios suitable for mapping in tetraploid alfalfa were 1,738 SNPs for simplex x nulliplex, 582 SNPs for duplex x nulliplex, and 426 SNPs for simplex x simplex. TetraploidMap software was used to integrate SNPs from the array with an existing framework map. The colinearity between the resulting linkage map and the *Medicago truncatula* physical map was evaluated. The resulting high-density linkage map in alfalfa generated from this study can be useful to guide the ongoing assembly of the tetraploid alfalfa genome sequence and to identify genic SNPs in relevant loci associated with key traits of agronomic importance. The alfalfa iSelect Infinium assay represents a platform for high-throughput SNP genotyping in alfalfa that greatly improves genotyping efficiency and enables detection of allelic dosage in a tetraploid species.

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Improvements in biotic and abiotic stress tolerance among cool-season turfgrasses

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Abstract

Population increases and urbanization are put increasing pressures on turfgrass recreational areas. The aesthetic and functional quality of lawns, parks, golf courses and athletic fields depend on the following characters which are usually assessed in turfgrass variety evaluation programs: establishment rate, density, leaf fineness, color (darkness of the green color), dormancy color (green vs. brown/wilted when turf is not growing), winter damage (incl. both abiotic and biotic damages), resistance to specific diseases, wear tolerance (after using wear machines) and growth rate (leaf extension). These aspects all contribute to the overall impression, which is usually the basis for ranking turfgrass varieties.

The authors of this paper are currently involved in three evaluation programs; (1) the GEVES (French) network which also include test sites outside France, and (2) the SCANTURF and (3) SCANGREEN networks which assess to what extent varieties are adapted to the Nordic climate zone.

Perennial ryegrass (PR; *Lolium perenne*) is the most important cool-season turfgrass species. For this species we have observed significant improvements in tiller density, leaf fineness and wear tolerance, as also reported by Sampoux et al. (2012). On the other hand, there has been little improvement in winter survival or drought tolerance, which are major constraints to more use of perennial ryegrass in northern or continental areas. Perhaps this will change as new tetraploid cultivars of PR are now coming to the turf marked.

For Kentucky bluegrass (KB; *Poa pratensis*) we have also seen significant improvements in density, leaf fineness and mowing tolerance. This justifies a role of new KB varieties in seed mixtures for tees and fairways cut at 9-15 mm, particularly in northern areas where summer temperatures are not too high but there is a risk for winter damages. However, as there has been little improvement in establishment rate, it is still an open question if it is worth the extra cost to include KB in seed mixtures of overseeding. Because less need for fungicides, fertilizer and water, we are seeing a trend for golf courses in shifting from bentgrasses (*Agrostis* sp.) to red fescues (RF; *Festuca rubra*). Within RF there are numerous breeding challenges with regard to establishment rate, density, winter color, tolerance to abiotic winter damages and competitiveness against weeds in the absence of herbicides and at low fertilizer inputs. So far, we have seen the most significant improvements in ssp. rubra,

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Evaluation of grass species cultivars and mixtures for use in high class lawn tennis courts

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Abstract

Tennis has been played on natural grass since the first lawn tennis club was founded in 1872. There has been an international tennis tournament played on grass at Wimbledon since 1877. Choosing grass species for this specialised use was initially determined by chance rather than design. The game was first played on ornamental lawns that were also used for croquet and the fine fescue (*Festuca* spp.) and bentgrasses(*Agrostis* spp.) associated with these lawns were the grasses of choice. In more recent times (1950s and 1960s) the development of grass breeding for sports and other amenity uses has improved other species which could be considered for and have been included in seed mixtures for this use; the principle species being *Loliumperenne*.

No specific investigations of variation among grass species and cultivars for intensive tennis management and use were attempted until a programme of studies were initiated at the STRI in 1993. The aim of this work was to identify improved grasses for inclusion in courts at the All England Lawn Tennis and Croquet Club. This paper reviews work completed as part of this programme. The first trial conducted evaluated five grass species (*A. capillaris*, *A. stolonifera*, *F. rubra*, *Poapratensis*and *L. perenne*). This included different subspecies of *F. rubra* and a range of cultivars for most species. Mixtures of different species with varying cultivar combinations within mixtures were also evaluated. Results demonstrated that three types of grass were tolerant of intense tennis type abrasive wear. These were *L. perenne*, *P pratensis* and to a lesser degree the slender creeping red fescue grouping of *F. rubra*. Choice of cultivar within these groups was also very important. Retained live grass cover after wear was between 1.5 and 2.0 times greater for the best cultivars compared with the worst in each group. Evaluation of the mixtures also demonstrated that cultivar choice, particularly for *L. perenne* was very important. A second investigation sown in 1994 produced similar results for grass species and cultivars sown as monocultures. However, the performance of *P. pratensis* in mixtures appeared to differ from the previous study and in relation to performance as a monoculture in the second investigation. The better cultivars sown as monocultures had comparable wear tolerance to the better cultivars of *L. perenne*. In mixtures of the two grasses *P. pratensis* appeared to perform poorly. The relative proportion of *P. pratensis* in mixtures declined markedly following wear. A third study was sown in 1998 with an expanded brief. The objectives for this study were to examine variation in surface properties as well as differences in durability among grass species and cultivars. The work demonstrated that choice of species could affect surface hardness and as a result of this ball bounce. Cultivars of *L. perenne* produced a harder surface than *F. rubra* and *P. pratensis*.

Breeding for 'healthy hay': Can we optimise plant polyphenols in legumesfor ruminant nutrition, animal health and environmental sustainability?

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Abstract

A small number of forage legumes are of great interest for sustainable livestock production because they contain various bioactive compounds, such as polyphenols, tannins and polyphenol oxidase. The recently completed EU Marie Curie Research Training Network ('HealthyHay') reported large intra-species variation not only in agronomic characteristics but also in these bioactive compounds during the detailed screening of a sainfoin (*Onobrychis viciifolia*) germplasm collection. Tannins, for example, showed considerable quantitative and qualitative differences: contents varied 5-fold, the average polymer size varied 7-fold, prodelphinidins accounted for between 53 and 95 % and *trans*-flavanols for between 12 to 34 % of the tannins (Stringano *et al*., 2012). Large variations were also observed amongst monomeric polyphenols (Regos*et al*., 2011) and between enzymes involved in the biosynthesis of flavonoids (Thill*et al*., 2012). Flavonols and flavan-3-ols were important variables for distinguishing germplasm accessions and dihydroflavonol 4-reductase and flavonol synthases proved particularly useful for screening purposes. Not surprisingly, considerable variation was also observed in terms of their effectiveness against parasitic worms, coccidia and ruminal fermentation characteristics.

This EU 'HealthyHay' project used existing and established several new techniques for screening bioactive legumes for bioactive compounds, but also for anti-parasitic properties, nutritional and environmental benefits. These included *in vitro* anthelmintic tests (exsheathment and larval feeding inhibition assays), an anti-coccidial test (oocyst sporulationasssay) and anautomated pressure evaluation system (APES) for volatile fatty acid and methane production.Further work will be needed to identify the optimum concentrations and compositions of various bioactive compounds and to develop molecular markers for this underexploited forage legume. Recent research on *Populus* indicated that the qualitative and quantitative tannin traits are under genetic control and thus amenable to plant breeding. More work will also be needed to optimise harvesting and preservation procedures in order to maintain the beneficial effects of sainfoin and other bioactive forage legumes.

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Can high-throughput genotyping be combined withfine-resolution environmental data for a more efficient use of natural diversity in forage and turf breeding?

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Abstract

European gene banks maintain collections of natural accessions of forage and turf species that all together may represent a wide-range sampling of the genetic variability available over a great part of the environmental niche of these species. The increasing availability of technologies for the efficient screening of genome-wide polymorphisms offers new opportunities to investigate genomic diversity and more specifically to search for genomic diversity involved in the response of populations to natural selection (Storz, 2005). In addition, environmental databases covering large spatial scales at fine resolution are now available, especially regarding climatic information. New high-throughput genotyping technologies and detailed environmental information can be combined to search for associations between genomic polymorphisms and environmental variation at sites of presence of the natural diversity (Holderegger et al., 2008). Furthermore, methods to detect signature of selection using dense genome-wide genotyping data have been developed from population genetics theory (Beaumont, 2005).

This methodological framework has been implemented for several years in ecological genetics studies aiming at understanding the role of adaptive diversity in a number of species. It is well suited for studies at the macro-environmental level, which is the relevant level to investigate adaption to climatic constraints. We suggest that this framework can be applied to gene bank accessions collected throughout the natural distribution area of forage and turf species, in order to provide a new kind of information to guide the use of natural diversity in breeding programs. The statistical associations that may be revealed between genomic polymorphisms and environmental variation could contribute to several objectives. These associations could first contribute to decipher the genetic determinism of natural adaptation. Second, they could provide a means to model the environmental distribution of different alleles at loci involved in adaptive diversity. Such models could be used to assess the resilience of adaptive alleles against environmental variationsand to foresee the spatial range shift of these alleles with climate change. Finally, this information could be used to determine novel sources of diversity to focus on in breeding programs and to facilitate their introgression in elite germplasm *via* the identified markers of natural adaptation.

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Fair evaluation of yield and quality potential in forage species evaluation

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Abstract

The evaluation of yield and quality in forage species is more laborious and time consuming and yet less precise than in cereals. Some reasons are well known, have been discussed frequently (multi cut and multi year trials) and will be difficult to overcome. In the last years another reason for the low repeatability of forage trials came into focus – the wide range of heading date.

The harvest date of forage plants is oriented towards a certain developmental stage.. According to this the varieties to be compared should be harvested at the same stage of development. Although this is very important for yield testing and even more important for quality assessmentit is often disregarded in the current practice. For practical reasons the tester harvests all entries in most forage species at one particular date. Only entries in those species which exhibit a very wide range in heading date the are divided into two, three or four harvesting groups. However, even within harvesting groups very different variety types may still be found. This is particularly the case in perennial ryegrass with a range of heading datesgoing up to 50 days for the species and to 14 days for a harvesting group.

In 2006 five breeding companies (DLF Trifolium, Barenbrug, Euro Grass Breeding , Norddeutsche Pflanzenzucht and Saatzucht Steinach) started a joint project with the German plant variety office (BSA). Its aim was to get more information about the influence of heading date on the yield and quality results in the existing testing systems. The project was carried out between 2008 and 2010 at five locations in Germany and The Netherlands. Yield data collection and processing was done like in a normal yield (VCU) trial by the BSA.. Additionally, samples for quality analysis were taken and analyzed in an ongoing second project supported by the German Agency for Renewable Resources (FNR).

This paper examinesthe difficulties of a fair evaluation of yield and quality potential, identifies the problems and points to possible solutions in order to overcome the most dangerous pitfalls.

Young researcher presentation

Monitoring, analysis and modeling of yield and quality dynamics of *Lolium perenne* varieties for biogas production

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Abstract

The overall aim of this project is to increase the biogas production of perennial ryegrass (*Lolium perenne)* by model-based optimization of the harvesting date. In a preliminary step it was analyzed statistically whether the harvesting date influences yield and quality of *L. perenne* varieties. In a second step a model for yield and quality development of the varieties was set up and parameterized.

In the first step 20 varieties of *L. perenne* differing in maturing times were cultivated at five sites in Germany and the Netherlands. Two harvesting treatments were applied: consistent harvest (H_c), where all varieties were harvested at the same time, as it is common practice, and differentiated harvest (H_d), where each variety was harvested at growth stage of ear emergence. Yield was characterized as dry matter yield, and quality parameters, such as crude protein and crude fiber, were determined by Near Infrared Spectroscopy (NIRS). Yield and quality data were analyzed descriptively and by ANOVA. It was shown that yield and grass quality varied mainly between site and year. A significant interaction between variety and harvesting treatment indicated, that yield and quality of both harvesting treatments differed particularly between the early and late varieties: Yield of early varieties was greater in H_c, while yield of late varieties was lower in H_c than in H_d. On the contrary, quality of early varieties was lower in H_c, while quality of late varieties was greater in H_c than in H_d.

In the second step, a logistic growth model for yield and quality development was set up, which also includes the most important environmental conditions, such as temperature and soil moisture. For model parameterization, eight *L. perenne* varieties with different maturing times were cultivated at four locations in Germany and harvested in time series. Yield and quality of *L. perenne* and environmental conditions were monitored. The model fitted well to both, yield and quality data of *L. perenne* varieties at all sites. The differences in yield and quality development were reflected by the model through differences in rates of growth and temperature coefficients: Early varieties had lower rates of growth and temperature coefficients than late varieties.

It can be concluded that this model can help to characterize *L. perenne* varieties and determine the optimal date of harvest for an increased biogas production.

Effect of alfalfa cultivar on pollinator visitation, seed yield and yield components

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Abstract

Three cultivars were used in the experiment. Two cultivars were developed at the Institute for forage crops (K-22 and K-28) and third was cultivar Medijana. Lot size was 2m^2, alfalfa was planted in rows and the distance between rows was 25 cm. The effect of cultivar on the visiting of pollinators (honey bees and other alfalfa pollinators), seed yield and seed yield components. To determine the visitation, the pollinators were counted in area of 1m^2 for 30 min. The yield was determined per square meter, and for examination of yield components 10 plants were sampled per replication and the counting was done on the lowest, middle and highest inflorescences. Most pollinators were attracted a by cultivar K-28 (13.2). Of that number, there were 9.13 honey bees and 4.07 other pollinators. Cultivar Medijana has attracted 9.81 and cultivar K-22 has attracted 9.06 pollinators. There were differences in terms of visitation caused by the time of counting. Most pollinators have been recorded in the mid-day counting. The relationship between honeybees and other observed pollinators was approximately 3:1 in favor of the honey bees. Considering seed yield, cultivar K-28 achieved 55.33 gm^{-2}, cultivar Median 47.28 gm^{-2} and cultivar K-22 yield was 46.94 gm^{-2}. Cultivar K-22 had the most flowers and fertilized pods in the middle and highest inflorescences as well as seeds per pod in the highest inflorescences. Cultivar Median had the most flowers on the lowest inflorescences. For other observed traits the highest values were determined in the cultivar K-28. By the percentage of fertilized pods at the highest inflorescences, the cultivar K-28 showed very significant differences from the other varieties. Considering the yield, number of flowers, number of pods and number of seeds per pod at the lowest inflorescences, the cultivar K-28 was significantly different from other cultivars. For other yield components differences between cultivars were not statistically significant.

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Young researcher poster

Condensed tannin analysis by thiolytic degradation in sainfoin (*Onobrychis viciifolia*) silage

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Poster No. 60

Abstract

Sainfoin (*Onobrychis viciifolia*) is a perennial forage known for its good nutritive value for ruminant animals. Its bioactive compounds arecondensed tannins.Sainfoin can be preserved as silage to provide energy and protein during periods of feed shortages; ensiling also appears to preserve condensed tannins. Fresh sainfoinwas ensiled in the laboratory for60 days with formicacid (8 ml/kgfresh weight) in plastic tubes.Condensed tannins (CT) were analysed after freeze-drying by thiolytic degradation with benzyl mercaptan (Gea*et al.*, 2011).Ensiling affected condensed tannin composition as follows:the apparent content of total CT decreasedfrom 2 to 0.9 g/100 dry matterand the relative proportion ofprodelphinidin tanninsincreased from 73 to 80 %, which was especially noticed in compositional changes of flavan-3-ol terminal units. The results demonstrated that fermentationinduced changes in tannin structure. An increase in prodelphinidins may have beencaused by oxidation (auto-oxidation or enzymatic oxidation), whereasformation of covalent linkages could explain the lower tannin contents as determined by thiolytic degradation.

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Effect of different rhizobacteria on alfalfa, *Medicago sativa* L. yield by inoculation of a preceding oats, *Avena sativa* L.

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Poster No. 61

Abstract

This study was conducted to test the possibility that oats, *Avena sativa* L. inoculation with plant growth promoting rhizobacteria (PGPR) can promote growth of alfalfa, *Medicago sativa* L. as a subsequent crop. PGPR represent bacteria that colonize the rhizosphere and plant roots and have ability to enhance plant growth by different mechanism; i.e. increasing N uptake (biological N fixation-BNF), phytohormone production (auxin, cytokinin), minerals solubilisation, nutrients mobility, production of siderophores that chelate iron and make it available to the plant root, controlling pathogens. Thus, PGPR are a promising alternative for mineral and organic fertilization. They are the potential tool for sustainable agriculture and the trend for the future. A pot experiment was carried out with unsterilized soil under greenhouse conditions. Oats plants were inoculated with strains belonging to *Sinorhizobium meliloti*, *Azotobacter* spp. as well as *Enterobacter* sp. and *Bacillus* sp. After oats harvest, alfalfa seeds were sown as the subsequent crop. The experiment was designed with eight inoculated treatments with 3 replications in completely randomised system. Inoculated treatments were compared with control uninoculated treatments. Influence of oats inoculation on alfalfa was presented through plant performances (shoot dry weight, total N content and P content in plant shoot). Results indicated that the growth of alfalfa plants was improved by oats inoculation with particular PGPR strains. The shoot dry weight of alfalfa was increased by approximately 20% over uninoculated control depending on the strain applied. The nitrogen content was also increased in alfalfa plants. Beneficial effect on yield and N-assimilation in alfalfa was obtained due to inoculation of oats plants with particular rhizobacteria.

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Traits explaining genetic ability of alfalfa to forage production in alfalfa-fescue mixtures

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Poster No. 62

Abstract

New interest is given to alfalfa, an important forage legume crop, because it can contribute to agriculture sustainability. Mixtures of alfalfa with grass species combine, in a single sward, the advantages of the two species: seasonal growth pattern, biochemical composition, low nitrogen input. Forage production, but also the survival of each species, result from the ability of plants to get the resources needed for their growth, among which light and nitrogen are of major importance. The present study aims at describing genetic variation for traits related to resource acquisition in alfalfa-fescue mixture. Forty six contrasting genotypes from ten alfalfa varieties and two genotypes of tall fescue (a forage type and a turf type) were planted in September 2010. An innovative design was used: for each pair of alfalfa-fescue genotypes, it included spaced plants, monocultures in dense conditions (plots of 7 plants, the target plant being surrounded by 6 plants, 7 cm apart) and alfalfa-fescue mixtures (plots of 3 alfalfa and 4 fescue plants, 7 cm apart), with 3 repetitions. Each genotype of alfalfa and the two genotypes of fescue were clonally propagated to establish the treatments. Four harvests were taken in 2011 and 2012. Dry matter of harvestable biomass, plant height and nitrogen content were recorded for the target plant and the surrounding plants. In analyses of variance, the effects of alfalfa genotypes and treatments on alfalfa target plant and whole plot biomass were highly significant. The interaction between genotypes and treatments was significant, showing that biomass ranking of genotypes depended on the treatments. The alfalfa genotype effect on fescue biomass was not significant in spring, but in autumn, fescue biomass was different depending on alfalfa genotype. Nitrogen status of fescue was affected by alfalfa genotype in autumn. Light interception seemed to be the major source of competition between species, this process of energy capture had effect on nitrogen acquisition of both alfalfa (fixation) and fescue (absorption). These results offer prospects to identify breeding criteria to improve the ability of alfalfa to be grown in mixture with fescue.

Fertilization value of early red clover (*Trifolium Pratense* L.) 'Jõgeva 433', washington lupin (*Lupinus Polyphyllus* Lind.) 'Lupi' and crimson clover (*Trifolium Incarnatum* L.) as green manure crops

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Poster No. 63

Abstract

In 2008-2011, field trials were carried out at the Jõgeva Plant Breeding Institute (58°45' N, 26°24' E) in order to identify the possibilities of using the early red clover cultivar "Jõgeva 433" (diploid), Washington lupin with Jõgeva breeding cultivar 'Lupi' and crimson clover material of Italian origin as green manure. The fertilization value of the above species was studied after autumn ploughing in the year of sowing and second year from sowing based on the yield and quality of yield of spring wheat "Vinjett" and barley "Inari". The in-ploughed biomass as well as its composition were recorded. By the time of ploughing the most abundant biomass has been grown by Washington lupin 'Lupi'. The root system of the species is well developed; roots can be as thick as fingers. The sod is easily ploughable, but the roots remain alive until next spring and the recovering lupin plants may interfere with the aftercrops.

Of the studied species crimson clover had the lowest fertilization value, the stubble and roots of seed plants that were ploughed in resulted only in 6-7% of extra yield of spring wheat in the following year. Crimson clover had no fertilizing aftereffect in the second year. The fertilization values of red clover 'Jõgeva 433' and Washington lupin 'Lupi' were more or less equal. Their statistically significant positive effect on the increase of yield of spring wheat and barley lasted for 3 years, on the quality of yield for 2 years. Red clover 'Jõgeva 433' that was ploughed into ground in the year of sowing gave the maximum extra yield of 27% in spring wheat compared to N 0 variant, and Washington lupin 'Lupi' respectively 23%. In the second year of aftereffect Washington lupin 'Lupi' gave in barley an extra yield of 26.8%, red clover 'Jõgeva 433' 10.1%, and in the third year 10.4 and 12.1% respectively. Green manure improved the contents of crude protein and gluten in spring wheat and that of crude protein in barley.

The fertilization value of all studied species was higher in the case they were sown without a cover crop.

Washington lupin 'Lupi' cannot be recommended to organic farmers, because without the use of herbicides the species may become a troublesome weed.

Phalaris arundinacea L.: Variations in seed shattering and related traits in a breeding collection.

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Poster No. 64

Abstract

Reed canary grass is a perennial forage grass species with a high biomass yield potential and a wide adaptability to both, dry and excessive wet growing conditions. Seed yield depends on yield components like number of plants per m^2, number of panicles per plant, number of spikelets per panicle as well as flowering time, seed setting, seed development and seed retention at the time of maturity. In many years seed shattering causes seed yield losses up to 90 % (Hermann 1975; Sahramaa, Hömmö 2000).

In this study seeds of five genotypes were treated with an alkylating agent in order to increase the variability of seed retention in the genotypes. The treated seeds were germinated under defined conditions. The seedlings were vernalized and cultivated in a field trial at the experimental station of the institute located in Etzdorf, Germany. In 2012, the development of plants of the first generation after treatment, particularly the development of inflorescences and seeds was investigated. Seed shattering was tested using an equipment, which provokes seed shattering in a reproducible manner.

This paper reports first results.

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Festulolium: Grasses offering a security against climate change and an environmental service

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Poster No. 65

Abstract

Grasslands comprise a third of all agricultural land world-wide and are dominated primarily with perennial plant species.Their persistency across seasons providesa suitableenvironmenttosupport and maintainvast and bio-diverse species' communities. In addition to providing a regular supply of valuable fodder for livestock agriculture,grasslands also throughtheir species' interactions andtheir significant land areacontribute an essentialenvironmental safeguard against climate change. These includestoring and regulating releases of C, nutrient and water, thereby mitigating greenhouse gas emissions, safeguarding water quality and supply, and reducing incidents of flooding.Grassland systems are dynamic;each will differ in efficiency in delivery of agronomic and environmental service,a consequence of their species' composition and the impacts fromdifferent climatic and edaphic stresses they encounter.Wecan influence the former and redesign grasslands for significant environmental benefits.

More than 500 species of *Festuca*have been identified, in combination having the requisite adaptations essential for survival under most climatic and edaphic extremes.Their widespread dispersal has been achieved through their capabilities for genetic change and genome reorganisation, and in particular through stabilising advantageous genome complements following species' hybridisation throughpolyploidy. All are related to *Lolium*and many hybridise naturally to produce*Festulolium*. Those with an adaptive gain may evolve subsequently to become distinct forms andgiven their owntaxanomic classification.

However as hybrids in the hemizygous state, chromosome pairing regulator(s)in *Festulolium* cease to function resulting in genome instability andimbalance. No current synthetic amphiploidcultivar is known to retain a balanced genome complement, a consequence of preferential transmission across generations of *Lolium*chromosomes over those of *Festuca*. However, the failure to achieve stable amphiploidydoes provide *Festulolium*hybrids with opportunities for extensive genome recombination and thereby opportunities forgeneintrogression. Alien *Festuca*genes in *Lolium*genomes generally retain function and are capable of enhancing trait expression, evenin excess ofboth their parent genomes and moreover retain this function over generations. The outcome is the opportunity to exploit the vast genetic diversity within the *Lolium-Festuca*genome complex and redesign grass phenotypes fit-for-purpose for a range of environmental services or adaptations.

The influence of individual and combined inoculants on development of alfalfa on acidic soil

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Poster No. 66

Abstract

In this study the influence of pre-sowing inoculation with individual and combined cultures of rhizobium (*Rhizobium meliloti*), azotobacter (*Azotobacter chroococcum*) and actinomycetes (*Streptomyces sp*.) on the parameters of the top parts of alfalfa plants (height, number of tillers and plant weight) was investigated. Also, the weight of plant root was determined. Analyses were carried out in the beginning of blooming stage. The experiment was conducted as two-factorial, where the influence of seven variants of microbial inoculation on plant features was examined on two alfalfa cultivars (K-28 and Syntéza 1). Control was a variant without inoculation. The experiment was carried out in the vessels, in semi-controlled conditions. For the study, the acidity of the soil was elevated (pH 4.77). The effect of pre-sowing inoculation on investigated parameters was dependent on the inoculation variants and cultivars. Highest plant height was achieved in cultivar Syntéza 1 using polyvalent inoculum that contained all of the species of microorganisms. By using this microbial variant, the biggest weight and number of tillers per plant was achieved in cultivar K-28. In this cultivar using the combined cultures of rhizobium and actinomycetes, the highest root weight was achieved.

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Section History – summary of the last 10 meetings

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Abstract

One task is to analyse the past meetings to present some statistical data like the no. of participants and no. of presentations. With one exception there were always more than 100 participants (avg. 115) from 20 or more countries (avg. 24) including non-European countries and participants. Local participants accounted for 14 to 50%. The no. of presentations (incl. posters) ranged from 45 to 88. At half of the meetings workshops were held.

Another important task is the distribution of presentations according to the species and topics over time. Though there is a large variation the main crops accounted for the majority of all presentations. If one splits further into cool season grasses and legumes a very interesting pattern over time occurs, reflecting the interests of the hosting country. Among the grasses perennial ryegrass is the the most prominent species. Among the small grain legumes *Medicago* species are predominant, followed by red clover.

At the 21th meeting in 1997 there were only two papers dealing with molecular tools. At the meeting in 2002 there were already 13 presentations (22%). At the last two meetings 30% of all presentations focussed on DNA-technologies and their application.

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