EUCARPIA

European Association for Research on Plant Breeding
Europäische Gesellschaft für Züchtungsforschung
Association Européenne pour l'Amélioration des Plantes

ABSTRACT BOOK

XXVIIth EUCARPIA SYMPOSIUM ON IMPROVEMENT OF FODDER CROPS AND AMENITY GRASSES

Copenhagen (Denmark), 19th to 23rd August 2007

SOLIDUM PETIT IN PROFUNDIS · UNIVERSITAS ARHUSIENSIS

DLF TRIFOLIUM
SEEDS & SCIENCE

Programme

Sunday, August 19

18:00 - 20:00 Registration, poster mounting, welcome reception

Monday, August 20

7:30 Registration, poster mounting

Opening session:

8:30 **Ian Max Møller**, DJF, Research director Genetics and Biotechnology

8:45 **Beat Boller**, ART, Chairman EUCARPIA Fodder Crop and Amenity Grass section

Session 1:
Seed production

Sponsored by

Chairpersons:
Birte Boelt, Ulrich Posselt

9:00 **Anders Mondrup**, DLF
Limitations in seed production

9:30 **Birte Boelt**, DJF
Seed yield components and their contribution to yield

10:00 Coffee break and poster viewing

10:30 **Danny Thorogood**, IGER
Towards resolving vegetative and reproductive growth conflicts in turfgrasses

11:00 **Grzegorz Zurek**, IHAR
Relation between seed yield potential and turf quality in Poa pratensis *L.*

11:20 **Susanne Barth**, TEAGASC *Variation in seed yield and other morphological traits in a collection of Irish* Lolium perenne *ecotypes and bred varieties*

11:40 **Bruno Studer**, DJF
Genetic characterisation of seed yield and its components in perennial ryegrass Lolium perenne *L.)*

12:00 **Peter Button**, UPOV
New developments in the international union for the protection of new varieties of plants (UPOV)

12:30 Lunch, poster viewing

Session 2a:
Applied genomics

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Chairpersons:
Christian Huyghe, Odd Arne Rognli

14:00	**Thomas Lübberstedt**, DJF *EU-project "Development of allele-specific markers for sustainable grassland improvement (GRASP)":* overview
14:10	**Oene Dolstra**, PRI *GRASP: Candidate gene isolation*
14:30	**Thomas Lübberstedt**, DJF *GRASP: SNP discovery*
14:50	**Isabel Roldán-Ruiz**, ILVO *GRASP: SNP validation by association studies*
15:10	**Niels Roulund**, DLF *GRASP: Technology transfer to plant breeding*
15:30	Coffee break and poster viewing
16:00	**Philippe Barre**, INRA *Association studies using synthetic varieties: case study of GAI gene and leaf length in perennial ryegrass*
16:20	**Lesley Turner**, IGER *Changes in allele frequency during phenotypic selection for leaf water-soluble carbohydrate (WSC) in an experimental population of perennial ryegrass*
16:40	**Roland Kölliker**, ART *Genetic characterisation of persistence in red clover (*Trifolium pratense *L.)*
17:00	**Hans Lellbach**, BAZ *Mapping of LmPc1, a major dominant gene conferring resistance to crown rust in* Lolium multiflorum.
19:00	Social dinner at LIFE

Tuesday, August 21

Session 2b:
Applied genomics

Chairpersons:
Mervyn Humphreys, Roland Kölliker

8:30 **Odd Arne Rognli**, UMB
Genetic analysis of seed yield components

9:00 **Rikke Bagger Jørgensen**, Risø National Laboratory
Co-existence with GM crops: grasses, clover and fodder beet

9:30 **Heidi Rudi**, UMB
SNP haplotypes at candidate gene loci associated with frost tolerance in Lolium perenne *L.*

9:50 **Klaus Dehmer**, IPK
SNP genotyping of a large Lolium *genetic resources collection and data analysis via a diversity studies toolkit*

10:10 Coffee break and poster viewing

10:40 **Ian King**, IGER
Comparative analyses reveal the major fraction of functionally annotated gene models in monocots are located in recombination poor/very poor regions of the genome

11:10 **Birgit Hougaard**, Aarhus University
Bridging model and crop legumes using legume anchor markers

11:40 **Michael Abberton**, IGER
Using translational genomics to underpin germplasm improvement for complex traits in crop legumes

12:00 **Puthigae Sathish**, ViaLactia
Perennial ryegrass improvement through Cisgenics

12:20 **Muriel Vandewalle**, ILVO
DNA marker assisted selection for yield and nutritional traits in Italian ryegrass

12:40 Lunch and poster viewing

14:00 - 17:00 Parallel workshops
Chairpersons listed

15:30 Coffee break

Workshop 1: Genetic resources: optimal management and quality assessment

Beat Boller, ART
Klaus Dehmer, IPK

- Better use by breeders
- Core collections
- *Ex situ* vs *in situ* conservation

Workshop 2: Conventional versus molecular breeding

Niels Roulund, DLF
Ulrich Posselt, LSA

- Genetic diversity
- MAS
- Breeding Methodology

Workshop 3: DUS and VCU – implementation of molecular markers?

Ulf Feuerstein, EGB
Isabel Roldán-Ruiz, ILVO

- VCU
- DUS
- EDV

17:00 - 18:30 Plenary: conclusions from the three workshops

18:30 - 19:00 Business meeting for EUCARPIA members / Poster removal

19:00 Free evening / Copenhagen tours

Wednesday, August 22

8:00 From LIFE: Excursion to Tystofte (variety testing) and Research Centre Flakkebjerg

12:30 Lunch in Flakkebjerg

14:30 Visit at DLF-TRIFOLIUM A/S

19:00 Conference dinner near DLF-TRIFOLIUM A/S

Sponsored by DLF TRIFOLIUM SEEDS & SCIENCE

Thursday, August 23

Session 3:
Forage quality and bioenergy

Sponsored by Graminor

Chairpersons:
Fred Eickmeyer, Iain Donnison

8:30 **Ulf Feuerstein**, EGB
NIR-spectroscopy of non-dried forages as a tool in breeding for higher quality– laboratory test and online investigations in plot harvesters

9:00 **René Gislum**, DJF
NIRS and chemometrics – exploration of grass forage quality

9:20 **Kevin Jensen**, USDA
Forage quality of irrigated pasture species as affected by irrigation rate

9:40 **Leif Skøt**, IGER
Association of candidate genes with flowering time and forage quality traits in Lolium perenne.

10:00 Coffee break

10:30 **Slobodan Katić**, IFVC
Genetic and seasonal variations of fibre content in lucerne

10:50 **Ulrike Anhalt**, TEAGASC
Identification of biomass QTL in a perennial ryegrass inbred derived population

11:10 **Claus Felby**, LIFE
Biomass for bioenergy - What are the ideal properties of biomass crops

11:40 **Iain Donnison,** IGER
Quality traits for bioenergy in grasses

12:10 Closing of meeting

Other meetings/workshops

Rust workshop:

August 19, 11:00 - 16:00, see separate announcement
(roland.koelliker@art.admin.ch)

AGROBASE workshop:

August 19, 16:30 - 18:00, see separate announcement
(ulf.feuerstein@eurograss.com)

European Lolium & Festuca Initiative (ELFIN) meeting:

August 21, 19:00 - 21:00, see separate announcement
(torben.asp@agrsci.dk)

NIRS Online Forage User Group (NOFUG) meeting:

August 21, 19:30 - 22:00, see separate announcement
(ulf.feuerstein@eurograss.com)

Description of exhibitors

DLF-TRIFOLIUM A/S

Roskilde, Denmark
http://www.dlf.dk

With an estimated market share of 20% worldwide, DLF-TRIFOLIUM is the world's biggest seed company within the breeding, production, sale and marketing of cool season clover and grass seed species.

The production of clover and grass seeds is mainly situated in Denmark, as the climate is favourable here and the growers are highly experienced in seed production. The company's strong position on the world market for clover and grass seeds can be attributed to an efficient production and logistics function, where the seeds are cleaned on modern machinery and the quality of the product is documented via analyses in our own ISTA-accredited and authorised laboratories.

Barenbrug

Royal Barenbrug Group, Oosterhout, The Netherlands
www.barenbrug.com

The Royal Barenbrug Group is a globally operating grass seed company. We specialise in plant breeding, seed production and international marketing of forage and turf grasses.

Research and Development in all major climatic zones around the world has resulted in a unique product line of high-quality speciality varieties. The company participates in research alliances with major universities and institutes. New innovations include the breakthrough of unique food-quality improving characteristics of grass in the production of dairy products.

CLF PlantClimatics GmbH

Emersacker, Germany
www.plantclimatics.de

CLF Plant Climatics is the European market leader in the supply of plant growth and entomology chambers. As the exclusive partner of Percival Inc. in Europe we offer a broad spectrum of chambers from a compact LED chamber to a large area multi-purpose Plant Growth Chamber.

We take care to listen and understand what you need before offering advice on which chamber could be most suitable for your research work. All the chambers we supply are installed and tested on site by our own fully trained technicians - all you need to do is open the door of the chamber and put your plants in.

Doriane Scientific Software Solutions

www.doriane.com

DORIANE publishes LABKEY™, the core concept of this software package is the management of experiment cycle on live organism for research teams who requires sharing, security and tracking at the level of the Research Department.

LABKEY™ is made of two product lines based on one integrated data architecture, one for the management of organisms, their genetic properties and market qualities, the other for the management of high throughput analysis and molecular markers production.

Breeding companies and vegetal production agronomic department working on farm, vegetable and flower crops use LABKEY™.

Doriane has the recognized know-how to drive, with the IT customer team, the whole installation process and has many testimonies of participation to increase the efficiency of industrial Research Department having stations and laboratories in several countries.

LI-COR Biosciences

www.licor.com

The LI-COR 4300 DNA Analysis System, is a third generation infrared fluorescence detection system ideally suited to the exacting demands of functional genomics especially TILLING® (reverse genetics), Ecotilling (SNP research), and AFLP®. LI-COR photosynthesis, carbon dioxide analysis and light measurement instruments are recognized worldwide for standard-setting innovation.

WINTERSTEIGER AG

Ried, Austria
www.wintersteiger.com

WINTERSTEIGER provides solutions for the mechanisation of agricultural research. That means complete product lines of machinery in a stable modular system with the option of transmitting the data for the purpose of research, breeding, yield trials and multiplications of all crops i.g. cereals, rice, maize, sunflowers, oil seed, legumes, fodder plants, vegetable seeds, clover and various grasses or special crops.

The services include:
- Advice concerning the technical equipment for research stations
- Training and technical service for agricultural research equipment

EURO GRASS Breeding GmbH & Co. KG

Lippstadt, Germany
www.eurograss.com

EURO GRASS Breeding is one of the leading companies in forage and turf grass breeding as well as in small seeded forage legumes. The main species are perennial ryegrass, Italian and annual ryegrass, timothy, meadow fescue, cocksfoot, kentucky bluegrass, red fescue and different clover species. International marketing and sales are carried out by Euro Grass, a joint venture of Deutsche Saatveredelung AG (Germany) and Hunsballe Frø (Denmark). Combining the strengths of these partners a powerful breeding network, large seed multiplication and efficient structures in sales, logistics and other services are built up to meet the customers' needs.

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Invitrogen provides products and services that support academic and government research institutions and pharmaceutical and biotech companies worldwide in their efforts to improve the human condition. Invitrogen provides essential life science technologies for disease research, drug discovery, and commercial bio-production. Known for quality and consistency, Invitrogen's products and services accelerate discovery by providing optimized, ready-to-use tools and processes. That means faster experimental results, as well as a better understanding of the molecule, system, or organism you're studying. For more information about Invitrogen, visit www.invitrogen.com

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www.medinova.dk

Medinova Scientific A/S mainly operates within four market segments: Cell Biology, Molecular Biology, Haematology and Microplate Technology.

We have suppliers in Europe and USA. The company has 7 employees in Copenhagen, Denmark. Our customers are mainly governmental and industrial biomedical research laboratories.

Partec GmbH

Münster, Germany
www.partec.com

Since more than 20 years Flow Cytometry plays an important role in Agroscience and Plant Breeding worldwide. The uniqueness of flow cytometric ploidy and gemome size analysis made FC an indispensable tool for research driven as well as commercial plant breeders. Flow cytometric DNA analysis allows monitoring DNA content and Ploidy level of high numbers of individuals at an early stage of plant development. **Partec GmbH** contributes to this field of application since its early beginning through the development of dedicated, robust and reliable instrumentation and reagent protocols and can claim today to be market leader in the use of flow cytometry in these disciplines.

The Danish Agricultural Council

Copenhagen, Denmark
www.landbrugsraadet.dk

The Danish Agricultural Council (the DAC) is the leading special-interest organisation for farmers and the food industry in Denmark. In 2006 our members exported agricultural products and equipment in the amount of EUR 14 billion and employed a total of 180,000 people.

SIGMA-ALDRICH

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Produces and sells a broad range of biochemicals, organic and inorganic chemicals and related products.

Abstracts for oral presentations

Limitations in seed production

Mondrup, A.
DLF Trifolium A/S

Seed yield components and their contribution to yield

Boelt, B.
University of Aarhus, Faculty of Agricultural Sciences, Department of Genetics and Biotechnology, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark.
Tel: +45 89993500; fax: +45 89993501; E-mail: Birte.Boelt@agrsci.dk

The yield components in grass seed crops are identified as: The number of reproductive tillers, the number of florets per tiller and their success in producing a seed (floret site utilisation, FSU) and seed weight.

The majority of species in grass seed production in Denmark require a dual flower induction to initiate reproductive growth, and the traditional Danish production system is to establish grass seed crops undersown in at cover crop (companion crop). The management of these double crops production systems has attained much focus in research i.e. determination of optimal cover crop species, seed rate, row distance, nitrogen fertilisation rates and distribution between autumn and spring etc. Data from these experiments have shown that in slow establishing species as smooth stalked meadow grass (*Poa pratensis*, L.) and red fescue *(Festuca rubra*, L.) a low number of reproductive tillers may restrict the seed grower in obtaining the optimum seed yield. However, due to the large number of experiments, knowledge is available to avoid a low number of reproductive tillers to restrict seed yield.

In a number of grass seed crops cultivation practice includes the application of a growth regulator to avoid lodging before flowering. It is assumed that grass seed crops without any lodging at flowering have a higher FSU and hence a higher seed yield potential. Thousand seed weight (TSW) is normally determined in the pure seed lot, and often only very small variation is found within the same variety. This may be due to the fact that some seeds are lost due to shedding before harvest, some are lost in the harvest operation and some are lost during cleaning. In general it is assumed that the seeds lost during harvest and cleaning operations are small ‘empty’ seeds, however, experiments are initiated to examine to what extent seed weight may be increased by cultivation techniques.

Towards Resolving Vegetative and Reproductive Growth Conflicts in Turfgrasses

Thorogood, D. and Armstead, I.P.
Department of Plant Genetics and Breeding, Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Ceredigion, SY23 3EB, United Kingdom.

Turf quality is entirely dependent on vegetative characteristics, yet to produce a commercially viable turfgrass variety, its seed production potential must be optimised. However selection for seed production traits that are dependent on preferable allocation of resources from vegetative to reproductive organs will inevitably be negatively correlated with turf performance. The proportion of florets that produce a seed, and seed retention are two reproductive traits that are independent of vegetative growth performance traits and are therefore worth targeting. This paper focuses on seed set in perennial ryegrass for which there is considerable variation. Our approach has been to simply identify major QTL in mapping families that influence seed setting ability. In particular, we wanted to know whether the QTL identified were determined by resource allocation factors, that might suggest some compensatory mechanisms which would lead to no increase in overall seed yield potential, or, more significantly, genetic breakdown of gamete/embryo development processes. By comparing orthologous regions in rice, we also wished to identify putative orthologues of genes underlying these QTL, the physiological basis of which may already be known.

Relation Between Seed Yield Potential and Turf Quality in *Poa pratensis* L.

Prończuk S., Żurek G.
Plant Breeding and Acclimatization Institute, Radzików, 05-870 Błonie, POLAND

The relation between seed yield and turf quality was disputable from many years. Also search for good 'marker' traits for seed yield and turf quality predicting is still in progress.

Two experiments: one for seed yield and second for turf quality were established in Radzików (Central Poland). Twenty seven entries (10 cultivars and 17 breeding strains) of Kentucky bluegrass (*Poa pratensis* L.) were tested during 2001 – 2004. Seed yield was estimated on the basis of following traits: seed yield per plot (SY), panicle seed yield (PY), seed heads per area unit (SH), panicle length (PL), 1000 seed weight (TSW), plant height (PH), leaf width (LW) and heading time (HT). Turf quality was estimated on the basis of: visual merit (VM), shoot density (SD), leaf fineness (LF) and color (C).

Tested entries were significantly different for all seed yield and turf quality traits. Negative and significant correlations were calculated for PY and turf quality traits (exc. C). No significant correlations were found between SY and VM. None of top quality turf varieties (BARCELONA, LIMUSINE and CONNI) yielded as high as the highest yielding entries (BALIN, BARON etc.).

The best marker trait for seed yield was PY and for turf quality – late HT and low PY.

Efforts should be made to improve seed yield components but only minor chances are to combine very good TQ with high SY in one variety.

Variation in Seed Yield and Other Morphological Traits in a Collection of Irish Lolium perenne Ecotypes and Bred Varieties

Barth, S[1], McGrath, S[1,2], Charles, MT[1], Zen, D[1] and Hodkinson TR[2]
[1]Teagasc, Crops Research Centre, Oak Park, Carlow, Ireland; [2]University of Dublin Trinity College, School of Natural Sciences, Botany Department, Dublin 2, Ireland

In general, grasses show a huge diversity in gross morphology. Several reproductive and vegetative traits are used as taxonomic descriptors for the description of species and for the distinctiveness, uniformity and stability (DUS) variety testing. Variability for both types of traits, reproductive and vegetative, is the prerequisite to improve these traits by breeding. In 2005 we used 2500 spaced plants derived from 50 *Lolium perenne* ecotypes and bred varieties to assess variation in reproductive and vegetative morphological traits. For all quantitative traits four measurements and for all qualitative traits one measurement per plant was taken. In a principal coordinate analysis 42.24% of the total morphological variation could be explained in the first two dimensions. We found surprisingly little variation within vegetative traits in the 50 *Lolium* forage accessions, but a large extent of variation for seed yield components. Average values for the in detail studied seed yield related traits rachis length, spikelet/spike, florets/spikelet and glume length were mostly highest in diploid and tetraploid bred varieties, but with the exception of the trait florets per spikelet. Rachis length was significantly correlated to glume length, number of spikelets/spike and florets/spike. Highly significant regression models could be built for seed yield related traits.

Genetic Characterisation of Seed Yield and its Components in Perennial Ryegrass (*Lolium perenne* L.)

Studer B, Jensen LB, Hentrup S and Lübberstedt T

University of Aarhus, Faculty of Agricultural Sciences, Research Centre Flakkebjerg,
Department of Genetics and Biotechnology, Forsøgsvej 1, 4200 Slagelse, Denmark

Seed yield is of major interest for the key grassland species *Lolium perenne* L. Although seed yield is a complex trait and affected by agricultural practices and environment, traits related to seed production revealed reasonable genetic variation and thus promises good prospects for improvement by selection. However, breeding progress for seed yield may be a trade-off to forage production and thus requires detailed characterisation of its underlying genetic components. An F_2 mapping population of perennial ryegrass recently characterised for vernalisation response (VrnA; Jensen et al. 2005) was assessed in the glasshouse for seed yield based on lattice designs with four replications over two years. The traits heading date, plant height, number of panicles, length of panicles, seed yield per panicle, flag leaf length, flag leaf weight and seed yield per plant revealed repeatabilities ranging from 41 to 76 %. The adjusted entry means and a genetic linkage map consisting of 31 SSR, 50 AFLP and 17 CAPS markers were used for QTL analysis. Of particular interest are two QTL on linkage group (LG) 1 and LG 2, each explaining 25 % of the observed phenotypic variation for the trait seed yield per panicle, which co-locate with QTL for total seed yield per plant.

New developments in the International Union for the Protection of New Varieties of Plants (UPOV)

Button, P.
UPOV, 34, chemin des Colombettes CH-1211 Geneva 20, Switzerland

UPOV, which continues to be the only internationally harmonized, effective *sui generis* system of plant variety protection, is continuing to expand. In December 2005, UPOV published a report on the impact of plant variety protection according to the UPOV Convention, some of the findings of which are summarized in this paper. With the expansion of UPOV in both geographical terms and in terms of the number of genera and species for which protection is sought, there are increasing demands for general information on the UPOV Convention. This paper explains some of the initiatives taken by UPOV in recent years to meet those needs, including the launch of a distance learning course and the development of new guidance documents on the examination of distinctness, uniformity and stability (DUS). One aspect of the UPOV Convention explored in this paper is the provision for essentially derived varieties. The relationship between initial varieties and essentially derived varieties is explained and the role of the authorities in matters concerning essentially derived varieties is considered. An overview of the current situation with regard to the possible use of molecular techniques in the DUS examination is also presented by reference to proposals considered within UPOV.

Development of ryegrass allele-specific markers for sustainable grassland improvement (GRASP)

Lübberstedt, T[1], Roulund N[2], Humphreys, M[3], Dolstra, O[4], Roldán-Ruiz, I[5], Posselt, U[6], Pašakinskienė, I[7], Barre, P[8], Rongli, O-A[9] et al. (see complete list of co-authors on: http://www.grasp-euv.dk)

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[4] Plant Research International B.V., Droevendaalsesteeg 1, 6700 AA Wageningen, The Netherlands.
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[6] University of Hohenheim, State Breeding Institute, Fruwirthstr. 21, D-70593 Stuttgart, Germany.
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[8] Institut National de la Recherche Agronomique, 86600 Lusignan, France.
[9] Department of Plant and Environmental Sciences, Norwegian University of Life Sciences, PO Box 5003, N-1432 Ås, Norway

GRASP was a EU framework V project involving eight public institutions and one commercial partner. The major topic of GRASP was the development of "functional" allele-specific markers associated with relevant traits in *L. perenne* such as forage quality, nitrogen use efficiency, disease resistance, and abiotic stress tolerance. More than 100 candidate genes for these traits were isolated and the respective alleles of 20 genotypes (*Lolium* test set = LTS) were sequenced in order to derive allele-specific DNA markers. In parallel, the LTS was used to develop synthetic populations to be propagated under divergent selection over two generations for the different traits of interest. The frequency shift of DNA marker alleles over generations was monitored and used to validate candidate gene – trait associations. In addition to markers and populations, genomic tools were developed including a BAC library for *L. perenne*, subtracted cDNA libraries, microarrays for expression profiling, and genetic reference maps. Information generated in GRASP will be made available through the existing forage grass database in the UKCropnet (http://ukcrop.net/grass.html). GRASP is expected to be a platform for modern forage grass breeding but also for international follow-up projects.

Identification of promising candidate genes for abiotic stresses and forage quality in perennial ryegrass

Dolstra[1], O, Koning-Boucoiran[1], CFS, Dees[1], D, De Vos[1], ALF
[1]Plant Research International B.V., P.O. Box 16, 6700 AA Wageningen, The Netherlands

An essential point of the EU-project GRASP was the choice of candidate genes for the study of allelic variation in perennial ryegrass (*Lolium perenne* L.) for genes relevant to abiotic stresses and forage quality. One route successfully applied in GRASP exploited gene sequence and function data from other species to find and pick up promising *Lolium* genes. The second route started with identification of promising *Lolium* genes. To this end, a GRASP cDNA microarray with 4200 genes in duplicate was generated, using clones from cDNA libraries enriched for genes affecting traits as cold tolerance, leaf area expansion in response to light and temperature, nitrogen-use efficiency, carbohydrate allocation and cell wall composition, and plant development. Comparative swap-dye experiments with various pairs of differently labeled total RNAs were subsequently performed to study the influence of environmental and genetic factors on gene expression in various plant tissues. The profiling of root tissue from plants grown at low and high nitrogen, for instance showed at high nitrogen significant overexpression for 175 genes and underexpression for 71. The corresponding figures for shoots from the same experiment were 34 and 124, respectively. In all, the gene profiling studies resulted in the identification of many trait-related candidate genes for the genetic studies envisaged.

Development of ryegrass allele-specific markers for sustainable grassland improvement (GRASP): SNP discovery

Lübberstedt, T[1], Xing, Y[1], Frei, U[1], Wollenweber, B[1], Schejbel, B[1], Asp, T[1], Roulund N[2], Humphreys, M[3], Dolstra, O[4], Roldán-Ruiz, I[5], Posselt, U[6], Pašakinskienė, I[7], Barre, P[8], Rongli, O-A[9] et al. (see complete list of co-authors on: http://www.grasp-euv.dk)

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[9] Department of Plant and Environmental Sciences, Norwegian University of Life Sciences, PO Box 5003, N-1432 Ås, Norway

GRASP was a EU framework V project involving eight public institutions and one commercial partner. The major target of GRASP was the development of "functional" gene-derived and allele-specific single nucleotide polymorphism markers associated with relevant traits in *Lolium perenne* such as forage quality, nitrogen use efficiency, disease resistance, and abiotic stress tolerance. For each of the candidate genes, allele sequences (minimum length 1000 bp per allele) of 20 diverse genotypes (*Lolium* test set = LTS) were sequenced in order to derive allele-specific SNP markers. For a total of 104 genes, allele sequencing was initiated. Allele sequencing was completed for 91 genes and have been used to derive informative SNP markers for selection experiments. At University of Århus, 11 expressed resistance candidate genes were analysed in detail, including 6 nucleotide binding site and leucine rich repeat (NBS-LRR) like genes and 5 non-NBS-LRR resistance gene analogues. The number of haplotypes per gene ranged from 9 to 27. On average one single nucleotide polymorphism (SNP) was present per 33 bp between two randomly sampled sequences for the 11 genes. NBS-LRR like gene fragments showed higher degree of nucleotide diversity, with one SNP every 22 bp between two sampled sequences. NBS-LRR like gene fragments showed very high nonsynonymous mutation rates, leading to altered amino acid sequences. Particularly LRR regions showed very high diversity with one SNP every 10 bp between two sequences on average. In contrast, non-NBS LRR resistance gene candidates showed lower degree of nucleotide diversity, with one SNP every 112 bp. 78% of haplotypes occurred at low frequency (<5%) within the collection of 20 genotypes. Low intragenic LD was detected for most R genes, and rapid LD decay within 500 bp was detected. Comparative analyses with regard to SNP density, heterozygosity, linkage disequilibrium, etc. across different gene families will be presented.

GRASP: SNP validation by association studies

Roldán-Ruiz, I[1], Barre, P[2], Brazauskas, G[3], Dolstra, O[4], Humphreys, M[5], Lenk, I[6], Lübberstedt, T[7], Pašakinskienė, I[3], Posselt, U[8], Rongli, O-A[9], Roulund N[6], Rudi, H[9], Turner, L[5], Van Daele, I[1].

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In this project we have established a methodology for the validation of effects of candidate genes in *L. perenne* populations. The approach is based on the detection of shifts in allele / haplotype frequencies in populations that have been subjected to divergent phenotypic selection during several cyles of recombination. In short, the methodology involves the following steps: (i) Selection of a set of genotypes with contrasting characteristics for the trait under investigation; (ii) Creation of a panmictic population based on these founder genotypes; (iii) Starting from this panmictic population, carry out divergent selections; (iv) Compare the allelic frequencies of candidate genes in the initial population, and the selected populations. Significant changes in allelic frequency between positive and negative selections with respect to the initial population are an indication that the gene investigated is involved in the determination of the trait. Given that *L. perenne* is an obligate outcrosser, changes in allelic frequencies were established using standard population genetics approaches. The main advantage of this strategy with respect to 'typical' QTL analyses in families, is that the effects of a larger number of alleles can be analyzed simultaneously. It is anticipated that the exploitation of knowledge generated in this kind of populations will be easier to extrapolate to unrelated genotypes than knowledge generated in families derived from two genotypes.

GRASP: Technology transfer to Plant Breeding

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In the course of the European GRASP project, nine partners collaborated in identifying single nucleotide polymorphisms (SNP) in nearly 100 genes from Perennial Ryegrass (*Lolium perenne*). These SNP markers can be used for plant breeding in three ways: 1: As a simple system with randomly distributed markers making use of marker-marker associations. 2: As a tool for marker development, when the identified SNP is located in a gene likely responsible for a specific trait, but the trait-specific allele of this gene is still unknown. Eventually the identification of multiple SNPs in this gene will enable the definition of functional allele(s). 3: As a functional marker. When the function of the gene is fully known and it is clarified which sequences of the gene is responsible for the desired phenotypes. The tree ways will be exemplified using data from the GRASP project and by setting up examples of breeding programmes.

Association Studies using Synthetic Varieties: Case Study of GAI Gene and Leaf Length in Perennial Ryegrass

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Identification of genome regions explaining quantitative trait variations is generally obtained from QTL analyses on mapping populations exhibiting a low allelic variability. An alternative could be association studies using collections of individuals. Any structure of these collections could hinder detection of associations. An ideal plant material for association studies would be multi-allelic and unstructured populations.

The objective of this study was to test whether synthetic varieties could be used for association studies.

In a first experiment, three perennial ryegrass varieties with contrasting number of parental plants were studied for their structuration, variability and linkage disequilibrium (LD) decline. This decline defines the association study approach to be envisaged: "candidate gene" when LD decreases rapidly or "genome scan" when LD decreases slowly. The varieties were not structured. LD decreased more rapidly when the number of parents increased. No LD existed beyond 150 bp for Herbie, a variety built from 336 parents whose molecular and morphological diversity was similar to a core collection.

In a second experiment, an association study was performed between the Gibberelic Acid Insensitive gene and leaf length in the variety Herbie. One specific SNP explained 5 % of leaf length variation. Differences between genotypic classes of this SNP reach 45 mm for a mean value of 312 mm.

Changes in Allele Frequency during Phenotypic Selection for Leaf Water-Soluble Carbohydrate (WSC) in an Experimental Population of Perennial Ryegrass.

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Carbohydrate content is an important component of forage quality with direct effects on nutritive value. Increasing carbohydrate content increases the utilisation of protein in the rumen, which in turn increases milk and meat production whilst concomitantly reducing nitrogen losses to the environment. C0 seed of 3 experimental populations was produced from an initial set of 20 genotypes of perennial ryegrass (the most important forage grass for temperate livestock production) as part of the EU GRASP project. One of these populations was used to phenotypically-select for high and low WSC over two generations, producing C2+ and C2- populations. A Syn3 reference population was also produced with no conscious selection. The C2+ and C2- populations had significantly different WSC content in spring 2007, with the Syn3 population being intermediate. Allele frequencies of both SNP and SSR markers were compared between the various populations. Shifts in allele frequency between the C2+ and C2- populations and the original synthetic (Syn1=C0) that could not be explained by genetic drift (assessed by comparing the original Syn1 population and the Syn3) indicated effects of selection. The map location of the markers with the greatest shift in allelic frequency has been compared with known WSC QTL positions.

Genetic Characterisation of Persistence in Red Clover (*Trifolium pratense* L.)

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Red clover cultivars improved for persistence may help maintaining optimal legume proportions in temperate grasslands and thus may substantially contribute to high forage yield and quality over several years. However, persistence is a complex trait, which is difficult to improve. Phenotypic or genetic markers closely linked to genes controlling complex traits may offer additional tools for breeding to complement traditional approaches. The objectives of this study were to optimise the phenotypic evaluation of persistence, to identify QTLs for this important trait and to investigate the association of persistence with other important traits. Plant vigour and various morpho-physiological characters were evaluated during four growing periods in a mapping population consisting of 280 F_1 individuals and QTL analysis was performed based on a genetic linkage map consisting of 42 SSR and 216 AFLP markers. A weighted average of vigour scores assessed over two winters and three growing season was identified as the optimal method to phenotype persistence. For this index, one QTL explaining 12.2% of the total phenotypic variation was identified. No negative correlation between persistence and seed yield was observed, but persistence was positively correlated to length of stem, which in turn was positively correlated to seed yield. The phenotypic and genetic markers identified in this study have the potential to assist future breeding efforts for the improvement of persistence in red clover.

Mapping of ***LmPc1***, a major dominant gene conferring resistance to crown rust in ***Lolium multiflorum***

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Crown rust caused by the biotrophic fungus *Puccinia coronata* f. sp. *lolii* is known as one of the most serious foliar diseases in Italian ryegrass. We were able to genetically identify a single dominant resistance gene in BC1 families of Italian ryegrass using detached-leaf tests and a crown rust population as inoculum. In total, 274 individual plants could be defined with respect to their genetic constitution at the resistance gene locus. We have named this locus *LmPc1* for ***L****olium* ***m****ultiflorum* resistance towards ***P****uccinia* ***c****oronata* **1**. Bulked segregant analysis allowed us to identify linkage between *LmPc1* and a single microsatellite marker, which has previously been mapped on LG2 in *Lolium*. A comparative-genomic approach based on DNA-sequence information of publicly available *Lolium*-ESTs and the rice genome data resulted in a set of novel STS-markers for *Lolium*. The probability of recombination between two flanking markers, which cover a genetic distance of 14.7 cM, and *LmPc1* is only 0.0047, if both markers are taken together as a single selection criterion. Results obtained so far reveal a degree of co-linearity between the *LmPc1* genomic region on LG2 and R4 at the genetic map level, which will allow to narrow *LmPc1* systematically down with molecular markers.

Genetic Analysis of Seed Yield Components

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Seed yield is a highly complex trait which is influenced both by genetical and environmental factors. In grasses seed yield can be divided into components like the numbers of fertile tillers, spikelets per panicle and florets per spikelet, which determine the seed yield potential, and fertility and 1000-seed weight, which determine the utilization of the seed yield potential. In addition, seed yield is influenced directly or indirectly by a number of agronomic traits such as plant height, leaf area, dry-matter yield, heading date, lodging resistance and proneness to seed shattering. Many studies of seed yield and its component traits have demonstrated large genetic variation and high heritability for these traits, and these estimates are larger than for vegetative production traits. The larger genetic variation for seed production traits is attributed to the lower selection pressure than for vegetative production during breeding. A major challenge in improving seed yield by breeding is environmental interactions, i.e. inconsistent correlations between estimates obtained in spaced plantings and in dense stands. Utilization of the seed yield potential may also be unpredictable in dense stands due to lodging and insufficient pollination, which make predictions from dense stands unreliable. The challenges of genetic analyses of seed yield components will be illustrated by the results from a QTL study of seed yield components in meadow fescue.

Co-existence with GM crops: grasses, clover and fodder beet

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In 2006 the global cultivation of genetically modified crops increased with 13% and reached 102 million hectares. The GM crops cultivated are mainly soybean, maize, cotton and oilseed rape, but other modified crops are appearing, e.g. in 2006 herbicide tolerant alfalfa was commercialized in US. Also in Europe, GM crop cultivation is increasing with the largest areas in Rumania and Spain followed by Portugal, France, Germany, Czech Republic and Slovakia. To ensure that farmers and consumers can choose freely what food they want to grow and eat, national co-existence rules are now being fixed by law.

The measures to keep GM crops and NON-GM crops separated will be crop specific, because the potential for gene dispersal differ among the species. Among the fodder crops, grasses from the genera *Lolium* (rye-grass), *Festuca* (fescue) and *Poa* (meadow-grass) that are outcrossing and wind pollinated will needed strong control measures to ensure co-existence. This is probably also the case for species of clover (*Trifolium*) that are outcrossing, insect pollinated and may have vegetative propagation and longevity of seeds in the soil seed bank. The fodder beet (*Beta vulgaris*) on the other hand seems to be a crop, where co-existence can easily be achieved.

This contribution will present results from *Lolium perenne*, *Trifolium repens* and *Beta vulgaris* on field experiments and mathematic modeling of gene dispersal; in the field experiments dispersal was analyzed using DNA marker techniques. Based on the results we discuss the steps necessary to ensure co-existence with GM varieties of these fodder crops.

SNP Haplotypes at Candidate Gene Loci Associated with Frost Tolerance in *Lolium perenne* L.

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The Lolium test set (LTS) of 20 diverse genotypes (EU project GRASP) was used to isolate allele sequences and single nucleotide polymorphisms (SNPs) for candidate genes associated with cold acclimation and frost tolerance. Two generations of divergent selections for high- and low freezing tolerance were established from a synthetic population constructed by inter-crossing five LTS genotypes. Genetic drift was monitored by comparing allele frequency shifts against shifts in a parallel random mating population. Haplotypes were identified using haplotype specific sequencing, and high throughput SNP genotyping of experimental populations performed using the MassArray platform. For one of the candidate genes, fructan 6-fructosyl transferase (*6ft*) gene, SNP genotyping defined five haplotypes (alleles) but only two parental haplotypes were observed in the divergent selections. One of the haplotype was fixed in population selected for high frost tolerance and selection may be the driving force behind the observed allele frequency shift. Results from the analyses of the complete set of candidate genes will be presented. Our results implies that the strategy used in this study, i.e. defining SNP haplotypes and studying their allele frequency changes under selection for desired agricultural traits, is a promising strategy for identifying functional markers for plant breeders.

SNP Genotyping of a Large *Lolium* Genetic Resources Collection and Data Analysis via a Diversity Studies Toolkit

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The *Lolium* collection maintained at the IPK Genebank contains more than 3,000 accessions, mainly from the species *Lolium perenne* and *L. multiflorum*. Within this material, wild species, ecotypes, landraces, breeding material, as well as cultivars are represented.
In an effort to make this huge collection more readily accessible to a broad range of potential users, SNP markers were developed, starting from IPK's barley EST resource. The genotyping of appr. 2,900 accessions was performed via pyrosequencing and SNP allele quantification of seed bulks. As a prerequisite for building a comprehensive analysis platform for genetic diversity studies, the results - together with passport and phenotypic characterization data on the respective accessions plus sequence data from the IPK Sequence Database - were compiled into a joint database. This is integrated into IPK's Plant Data Warehouse and combines all data in an application specific "Diversity" data mart. Based on this data storage unit, the Diversity Studies Toolkit (DiSTo) for different data queries, comprehensive and multivariate data analysis, and result visualisation was developed. DiSTo provides different features including (i) accession filtering, (ii) colouration/labelling of selected accessions and/or groups, (iii) genetic distance calculation, (iv) phenogram construction, and (v) principal component analyses together with multidimensional visualisations.

Comparative analyses reveal the major fraction of functionally annotated gene models in monocots are located in recombination poor/very poor regions of the genome

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Sequencing of the rice genome has allowed an in depth analysis of genome organization in a model monocot plant species. This has provided a powerful tool for genome analysis in large genome un-sequenced agriculturally important monocot species such as wheat, barley, rye, *Lolium* etc. Previous data has indicated that the majority of genes in large genome monocots are located towards the ends of chromosomes in gene-rich regions which undergo high frequencies of recombination.

Here we demonstrate that a substantial component of the coding sequences in monocots is localized proximally in regions of very low and even negligible recombination frequencies. The implications of our findings are that during domestication of monocot plant species selection has concentrated on genes located in the terminal regions of chromosomes within areas of high recombination frequency. Thus a large proportion of the genetic variation available for selection of superior plant genotypes has not been exploited. In addition our findings raise the possibility of the evolutionary development of large super-gene complexes that confer a selective advantage to the individual.

Bridging Model and Crop Legumes using Legume Anchor Markers

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Comparative genetic mapping allows the transfer of genetic information between species. In the legume family the genomes of a few species (e.g. *Lotus japonicus*) are well characterised, and genome information from these can contribute important information to breeding programs in crop legumes with less well-characterised genomes. One recent example is the development of molecular markers tightly linked to the virus resistance gene *Rsv4* in soybean using sequence information from *Lotus japonicus* (Hwang et al, Genome 49:380-388).

In a general effort to bridge the maps of crop legumes with those of model legume plants we have established an automated bioinformatic pipeline that suggest intron-spanning primers for use throughout the legume family. The primers are suggested from an alignment of orthologous genes from related species. To ensure that the markers represent true anchor points between the individual maps, the regions amplified correspond to single copy genes. The bioinformatic pipeline has so far yielded a total of 459 legume based primer sets (http://cgi-www.daimi.au.dk/cgi-chili/GeneticMarkers/table) and 1335 primer sets for the grasses (http://cgi-www.daimi.au.dk/cgi-chili/GeneticMarkers/grass). A number of the legume based primer sets have been tested in common bean (*Phaseolus vulgaris*) and the distantly related groundnut (*Arachis*) and comparative map studies are progressing.

Using Translational Genomics to Underpin Germplasm Improvement for Complex Traits in Crop Legumes

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This project was recently funded under the EU ERANET Plant Genomics initiative. The consortium consists of four partners in three countries. The objective is to create a robust physical map of the diploid red clover (*Trifolium pratense*), which will be anchored to the genomic sequence of the legume reference species *Medicago truncatula,* and aligned to the clover genetic map. The anchored physical map facilitates dissection of biological traits, future genetic improvement and marker assisted breeding in this important legume crop. The project will allow comparative analysis across legume species and create a model for translational genomics in crop legumes. Fingerprinting and end-sequencing of BAC clones (which is carried out by the Arizona Genomics Institute, USA) from an existing red clover library is used to obtain 2000 BAC contigs and anchor them to the *M. truncatula* genome using the closest homologue. Cytogenetics is used to assess the level of coverage of the clover physical map and resolve issues with misaligned contigs. Around 100 gene specific molecular markers previously tested in clover will permit an approximate positioning in the *M. truncatula* genome to be determined. A web accessible clover information resource with the alignment to *M. truncatula*, and integrated with the alfalfa resource developed in the US will be established. Integration with other databases will allow us to derive conserved orthologous sequence markers from the clover end-sequence tags that can be integrated with their counterparts in *M. truncatula* and *Lotus japonicus*.

Perennial Ryegrass (*Lolium perenne* L.) Improvement Through Cisgenics®

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Perennial ryegrass (*Lolium perenne* L) is a major grass species used for forage and turf throughout the world. The yield improvements in perennial ryegrass dry matter production achieved annually in New Zealand farms between 1961 and 1999 was less than 0.5%, and it reflects a yield plateau. It is our belief that the development of cisgenic® ryegrass will advance our long-term product development plans to improve animal productivity by providing pasture cultivars with traits of interest to industry, such as drought tolerance, increased biomass, increased persistence, increased pasture quality, and increased condensed tannin content. Our functional genomics platform consists of markers based on Gene Thresher® technology (Gill, *et al.*, 2006), ryegrass SAGE™ (Sathish *et al.*, 2007) for transcriptome analysis and functional testing of genes in ryegrass and rice. We have developed a high throughput *Agrobacterium tumefaciens* mediated transformation method of perennial ryegrass (Bajaj *et al.*, 2006) to enable us to identify the ryegrass genes for our target traits. We have isolated the ryegrass homologue of *Atvp1*, *Lpvp1*, and produced transgenic plants driven by constitutive double strength CaMV *35S* promoter. Our preliminary experiments on T0 plants suggest high drought tolerance in some lines. After identifying the target genes, we have replaced *Agrobacterium* T-DNA sequences with ryegrass derived ‘P-DNA’ sequences for *Agrobacterium*-mediated transformation. We have also produced transgenic ryegrass plants over-expressing *Lpvp1* driven by perennial ryegrass derived constitutive or inducible promoters. As part of our Cisgenics® based functional genomics platform, we are now assessing *Lpvp1* and other Cisgenic® genes as selectable markers.

DNA marker assisted selection for yield and nutritional traits in Italian Ryegrass (*Lolium multiflorum*)

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Our project aiming for the application of DNA MAS for yield and nutritional quality traits in the breeding of Italian ryegrass has been finalized. F1-populations generated by pair-crosses between non-related elite clones were used for the identification of QTL markers. MAS was performed on genotypes issued from the self-pollination of elite F1-genotypes selected on a phenotypic basis. Although, a ‘virtual’ (not effectively performed) MAS of F1-genotypes led to a mean for the traits higher than the F1-population means. In the S1-populations, MAS was complicated by the presence of new allelic combinations, absent at the F1-level. The different MAS compositions, obtained after intercrossing of the selected S1-genotypes, were evaluated in yield and quality plots. Results of the MAS selections were in the same range as the standard varieties and the traditional breeding compositions of same origin. Plants out of the self-pollinated populations, not retained by MAS and intercrossed together, consistently had lower results. Significant differences were observed between the two experiment locations. Thus, the gain obtained by MAS was not as expected and no significant heterosis effect was generated. MAS allowed overcoming the negative effect of selfing, however, no real difference was observed between negative and positive selections. It can be argued if the MAS applied was more effective in selecting loci with significant genetic variation than differentiating positive and negative alleles. The possibility of MAS for complex traits such as DMY and quality traits from a whole season observations in a very heterogeneous species like ryegrass will be discussed.

NIR-Spectroscopy of non-dried forages as a tool in breeding for higher quality – laboratory tests and online investigations in plot harvesters

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Near infrared spectroscopy (NIRS) implemented directly on plot harvesters represents an important challenge for the rationalisation of field trials in plant breeding and variety testing. Its aim is to assess the content of dry matter, protein, soluble carbohydrates and cell wall parameters of forages in real time during harvesting without having to expend resources on handling, drying, grinding and chemical analysis of samples. The bench mark for online measurements in the field is defined via laboratory tests using conventional NIRS apparatus on forages in the fresh undried state as well as in the dried and ground state. The state of the art achieved with a new type of sample presentation in the diode array spectrometer module of Haldrup A/S is presented. It is shown that NIRS employed in routine field testing allows the replacement of conventional DM testing by drying oven. Savings in labour and conventional equipment permit a depreciation of the investment within one to two years depending on the extent of performance testing at the breeding station. Finally, a strategy is advanced which may allow the sharing of the developed methodology with public institutions involved in variety testing.

NIRS and chemometrics – exploration of grass forage quality

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Near infrared spectroscopy (NIRS) has been successfully implemented as a method to quantify forage quality parameters e.g. crude protein in grasses. NIRS spectroscopy is basically an indirect method, and the spectra are essentially non-specific, hence, different constituents have broad overlapping peaks. For this reason NIRS measurements have to be calibrated against samples of known chemical composition, and the success of the NIRS method is therefore closely linked to the use of chemometrics.

NIRS is particularly well-suited for quantification of fats, proteins, carbohydrates and moisture, but other more sophisticated NIRS calibration models have been tested worldwide. The aims of this abstract are to describe the successful NIRS calibration model we have on total nitrogen and evaluate the development of new NIRS calibration models on lignin and ergovalin with focus on chemometrics and why we conclude that the lignin and ergovalin calibration models are not robust enough.

Forage Quality of Irrigated Pasture Species as Affected by Irrigation Rate

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The USDA-ARS Forage and Range Research Lab has initiated a breeding program to develop improved pasture grasses of tall fescue (*Festuca arundinaceae* Schreb.), orchardgrass (*Dactylis glomerata* L.), and meadow bromegrass (*Bromus riparius* Rehm.) that are productive and possess high quality forage for semiarid pasturelands, which are characterized by extreme temperature fluctuations and limited water resources. An understanding of how levels of irrigation affect crude protein (CP), neutral detergent fiber (NDF), digestible NDF (dNDF), and in vitro true digestibility (IVTD) are critical in pasture forage management. Cultivars of tall fescue, orchardgrass, meadow bromegrass, and perennial ryegrass (*Lolium perenne* L.) were established under a line-source irrigation system to evaluate the effect of five water levels (WLs) and multiple harvest dates on CP, NDF, dNDF, and IVTD. Within all species, the most notable trend across WLs was a near linear increase in CP going from the wettest to the driest WL. Orchardgrass maturity (early vs. late) had little effect on forage quality across WLs. Tetraploid perennial ryegrass cultivars averaged higher concentrations of CP, IVTD, and dNDF and lower NDF values compared with diploid cultivars. Endophyte-free cultivars of tall fescue had lower NDF and higher IVTD concentrations than their endophyte-infected counterparts at the higher WLs.

Association of Candidate Genes with Flowering Time and Forage Quality Traits in *Lolium perenne*.

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Association mapping is complementary to conventional F_2 or backcross mapping families. It uses populations of unknown pedigree to exploit the recombination events that have occurred over many generations, enabling a more refined mapping than is usually possible with conventional mapping families. We have used 864 genotypes in nine populations in a candidate gene approach for associating single nucleotide polymorphisms (SNPs) with variation in flowering time and water soluble carbohydrate (WSC) and other forage quality traits in *Lolium perenne*. Firstly, a within population association analysis was performed, as there was no evidence to suggest any genetic substructure within populations. Secondly, a linear mixed model was used on the whole data set. This enabled a structured association analysis to be performed, in which the clusters identified in the structure analysis to be incorporated as random variables. Thirdly, a Tree Scan method was used, in which haplotype trees based on inferred haplotypes, were associated with phenotypes. We found associations between several allelic variants in an alkaline invertase gene (*LpcAI*) and WSC, nitrogen and dry matter digestibility. None of them were detected in material harvested from both years. In contrast, we found consistent associations between an allelic variant (C4443A) located 265 bases upstream of the coding start site of the flowering time gene *LpHD1* and heading date. The usefulness of the C4443 marker is being evaluated in test crosses of genotypes from this work with genotypes of a turf-grass variety. The results of this experiment will be presented.

Genetic and Seasonal Variations of Fibre Content in Lucerne

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The digestability (quality) of lucerne depends on its fibre content. Its level is dependant on the stage of lucerne development and environmental conditions and on genetic factors (genotype) as well.

The objective of this paper was to determine the levels of cellulose, neutral detergent fibre (NDF), and acid detergent fibre (ADF) in five lucerne varieties developed at the Novi Sad institute for different purposes and using different breeding methods. The cellulose content varied significantly according to cut and was the lowest in the cooler part of the year (33.13%, 1st cut) and highest in the warmer one (41.36%, 3rd cut). Lower raw cellulose contents were found in the varieties Danka and NS Alfa. The NDF content was also the lowest in the 1st and highest in the 3rd cut (41.19 and 50.83%, respectively). The lowest NDF content and highest animal intake were recorded in the variety Danka (42.54%), followed by NS Alfa (44.69%) and then NS Mediana ZMS V (44.25%). The highest NDF content was found in Nijagara (45.54%) and Banat VS (45.05%).

The lowest (32.09%) and highest (40.93%) ADF contents were also recorded in the cooler and warmer parts of the year, respectively. The lowest ADF content was measured in NS Mediana ZMS V (34.81%) and the highest in NS Alfa. The variety Nijagara had the highest hemicellulose content (NDF-ADF), while Danka and NS Alfa had the lowest (7.66 and 7.69%, respectively).

Identification of Biomass QTL in a Perennial Ryegrass Inbred derived Population

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A genetic map of perennial ryegrass (*Lolium perenne*) was constructed from 73 SSR and AFLP markers based on 360 individuals of an inbred-derived F2 population. The F1 showed strong heterosis for biomass. We aimed to map quantitative trait loci (QTL) for biomass heterosis on the F2 population. An alpha lattice design was used for both greenhouse and field experiments. In the greenhouse three independent replicates were planted as single plants and three harvests were done from the experiment. In the field, the genotypes were planted in mini swards in two replicates and two harvests were carried out in 2006. Three major QTL for dry weight biomass were identified on linkage groups (LG) 2, 3 and 7, respectively. On LG 2 a QTL with an explained variance of 10% (LOD 4), on LG 3 of 19% (LOD 11) and LG 7 of 11% (LOD 8) were found. Heritabilities were reasonably high at about 70%. QTL for biomass is an important agronomic trait and increased biomass is still one of the most important traits in perennial ryegrass breeding programs. This study gives an indication of the position of biomass QTL and will benefit future molecular breeding programs.

Biomass for Bioenergy -What are the ideal properties of biomass crops

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The use of biomass for energy has become of strategic importance in order to secure the energy supply and reduce the emission of greenhouse gases, and biomass is now used for heath & power generation and substitution of diesel and gasoline with vegetable oils and ethanol.

The required properties for bioenergy are on a number of issues different from food and feed applications. The one major importance for biomass crops is high total yield. This implies that carbohydrates should be the main parameter for development of biomass crops. If the biomass is to be processed to fermentable sugars, a low lignin content has been found advantageous.

The biodegradability for biomass crops should be high, but also storage must be possible for at least 10 months in order to secure the energy supply and reduce the cost of conversion. Another issue is that low protein contents are preferred for, but at the same time biomass crops are an important source for animal feed.

Therefore the increased use of biomass for energy presents a number of challenges for our existing crops and extensive breeding programmes are required in order to exploit the full potential.

The presentation will present the parameters that should be included in the perfect biomass crop ideotype, and also a short review of current experience within breeding for biofuels will be presented.

Quality Traits for Bioenergy in Grasses

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The potential of grasses for energy is limited because plant varieties have not been selected for this purpose. There are distinct challenges to determine and improve quality traits to increase ultimate energy yields. Perennial grasses offer the potential to be utilised through either thermal or biological conversion methods to generate heat, electricity or transport fuels. The route chosen being largely determined by the calorific value, moisture content and the availability of carbohydrates. Chemical composition underlies these characteristics and can be measured to associate phenotype to genotype. For such studies it is necessary to develop both molecular markers in candidate genes and high throughput methods for phenotyping of composition. In the EU project GRASP, SNP based markers have been developed in carbohydrate associated genes which map to soluble carbohydrate QTL and these have been used in association studies in a synthetic population of perennial ryegrass to measure allele shifts. Furthermore infrared spectroscopy methods, calibrated with wet chemistry, have been developed in a number of energy grasses to determine lignin, cellulose and hemicellulose contents. These calibrations have allowed a comparison of chemical composition from different grass genotypes, species and environments.

Poster Abstracts

In Alphabetical Order

(First Author)

P01: Responses Seed and seedling genotype of Bromus, Agropyron to Fusarium avenaceum and F. graminearum

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This study was carried out to evaluate seed and seedling responses of genotypes of, *Bromus* (3 genotype) and *Agropyron* (3 genotype) to seed inoculation with spores of *Fusarium avenaceum* and *Fusarium graminearum*. Seeds of each genotype were inoculated with either of 2 levels of spores, 124×10^4/ml and 62×10^4/ml for *F. avenaceum* and 140×10^4/ml and 70×10^4/ml for *F. graminearum.*

Seed were grown under laboratory and natural conditions and non-inoculated seed was used as control. Percent and speed of germination, length of root and shoot, and vigor index were measured and responses of inoculated seed were compared with those of non-inoculated seed of each genotype.

There were significant differences among ecotypes for seedling characteristics due to the infection agents and concentrations. The effect of *Fusarium avenaceum* and *Fusarium graminearum* was more pronounced on speed of germination and vigor index than on germination percentage and other measured characteristics.

P02: Linkage Disequilibrium and Associations with Forage Quality at Loci Involved in Monolignol Biosynthesis in Breeding Lines of European Silage Maize (Zea mays L.)

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During recent decades, breeding efforts have lead to a substantial increase in whole plant yield of silage maize. However, during the same period of time there has been a steady decrease in cell wall digestibility, and, consequently, in feeding value of elite hybrids. Cell wall digestibility is influenced by both lignin content and lignin structure. Thus, genes involved in the lignin biosynthetic pathway are considered promising candidate genes for improving digestibility of silage maize.

Partial genomic sequences of 10 genes involved in biosynthesis of monolignols have been obtained in a number of inbred lines currently employed in European silage maize breeding. Different levels of nucleotide diversity and linkage disequilibrium (LD) were found, indicating different levels of selection pressure on individual genes of the monolignol pathway. Individual polymorphisms were tested for association with four quality-related traits to identify candidate functional markers for forage quality. Significant associations were identified, both when including and excluding population structure in the analysis. However, discrimination of effects of individual polymorphism was in some cases not possible due to extended LD. Studies in larger and/or broader sets of maize germlasm could decrease LD and validate candidate functional markers for forage quality identified in the present study.

P03: Vernalization Response in Ryegrass Involves Orthologues of Wheat *VRN1* and Rice *Hd1*

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Flowering time is important when adapting crop plants to different environments. In the breeding of perennial ryegrass (*Lolium perenne* L.) a dual requirement for flowering time exists. Flowering should be repressed to produce high-quality grass for feed while flowering should also be inducible to facilitate grass seed production. Consequently, the identification and characterization of the genes controlling flowering time in perennial ryegrass is of great interest.

Three candidate genes for vernalization response genes in perennial ryegrass were identified based on DNA sequence homology to *TmVRN1* and *TmVRN2* of diploid wheat (*Triticum monococcum*), and *Hd1* of rice (*Oryza sativa*). High sequence similarity between *LpVRN1* and *TmVRN1*, co-localization of *LpVRN1* with a major quantitative trait loci (QTL) for vernalization response in perennial ryegrass, synteny between map-positions of *LpVRN1* and *TmVRN1*, mRNA expression analysis of *LpVRN1* alleles during vernalization, and the correspondence between *LpVRN1* mRNA expression levels and flowering time leads us to conclude that *LpVRN1* is orthologous to *TmVRN1* and that its function is conserved between diploid wheat and perennial ryegrass.

Of the remaining two candidate genes, a putative *Hd1* orthologue, *LpCO*, co-localized with a second QTL for vernlization response. *LpCO* has recently been shown to be involved in the photoperiodic regulation of flowering time. While epistasis, at the level of *LpVRN1* transcription, was observed between the *LpVRN1* and *LpCO* genomic regions, no differential expression of *LpCO* transcripts was observed during vernalization. While orthologous genes controlling flowering time can thus be identified, future allele sequencing efforts will reveal if causative polymorphisms are conserved across the grasses.

P04: The DAFGRI Genome Project – Completed

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Perennial ryegrass (*Lolium perenne*) is one of the major temperate forage and turf grasses worldwide. An expressed sequence tag (EST) discovery program of randomly selected clones from 15 cDNA libraries representing three genotypes, a range of plant organs, developmental stages, and environmental treatments has been undertaken as part of the Danish Functional Genomics Research Initiative (www.dafgri.dk).

Approximately 30.000 ESTs have been generated, analysed by BLAST searches, and categorised in terms of their associated biological processes, cellular components and molecular functions using the Gene Ontology vocabulary. A non-redundant set of approximately 10.000 genes was identified from the EST collection, and used to develop a high-density cDNA microarray for gene expression analysis.

The EST sequences have been analysed to identify gene derived SSR and SNP markers, and aligned with the rice genome for comparative genomic studies. A BAC library consisting of 101.000 individual clones with an average insert size of 100 kb which constitutes five genome equivalents have been generated.

The resources are integrated with mapping populations and an efficient transformation system for evaluation of candidate genes for traits of interest.

P05: Comparison between two breeding methods in tetraploid perennial ryegrass: polycross versus F2

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Most of the perennial ryegrass cultivars are synthetics, composed of at least 3 (up to 20 or more) clones. The more components the more the risk of heterogenity. Is it possible to create highyielding varieties based on only 2 components?
To be able to compare synthetics with F2 populations based on the same starting material we created two synthetics, one based on 3 and the other on 4 selected clones. We carried out pair-crosses (F1) with the components within each synthetic (respectively 3 and 6 combinations), We also produced inbred lines (I1) of the components. The syn1, F1 and I1 were multiplied to the next generation (syn2, F2 and I2) in 2003. The progenies have been sown in a herbage yield trial in 2004. In 2005 and 2006 five and four cuts resp. were harvested.
There was no significant difference in herbage yield between the F2 and syn2 populations. Even the herbage yield of most of the multiplied inbred lines was not significantly different from the multiplied pair-crosses. The seed yield of all the F2 and I2 populations was lower than the syn2.
These results confirm roughly the previously reported results of identical trials carried out with diploid perennial ryegrass. However the inbreeding effect in the F2 and I2 populations on the herbage yield was less clear at the tetraploid level.

P06: Marker-Assisted Selection to Accelerate Genetic Gain in Open-pollinated Forages

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The use of marker-assisted selection (MAS) to accelerate genetic improvement of forages is often promised to revolutionise the breeding industry. However, realistic assessment of breeding targets, population composition, progression of the breeding cycle, trait genetic architecture, marker data collection and integration strategies, selection strategy, freedom to operate, and cost benefit ratios are essential for success. White clover (*Trifolium repens* L., 2n=4x=32) is an open-pollinated, high-quality, perennial forage legume with complex inheritance of traits underpinning pasture persistence, seed production, animal productivity, and animal health. The legume improvement programme at AgResearch has utilised seed production in white clover as a case study in the application of microsatellite markers for MAS of open-pollinated forage species. Selection for seed yield is the final hurdle in our recurrent selection programme for improved grazing and agronomic performance in white clover. A major QTL effect at the distal end of white clover linkage group D2 have been used to document frequency and extent of marker:trait associations in multiple complex breeding populations across three seasons, to create and evaluate genotypic selection criteria in experimental polycrosses, and to drive candidate gene identification and marker development. Experiments to utilise MAS selections earlier in the breeding cycle will be reported on, with a focus on maximising composite genetic gain by strategic use of MAS for seed yield with reference to the success criteria identified above. The presentation will include the first field data from spaced-plant evaluation of two series of MAS crosses in commercial white clover breeding populations, harvested April 2007.

P07: Improved Nitrogen Utilisation by Reducing Plant-Mediated Protein Degradation

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Inefficient utilisation of grazed forage protein in the dairy rumen is due in part to rapid protein degradation, and results in nitrogen (N) losses at energetic and environment costs. Exploitation of variation among forages for rate and extent of plant-mediated protein degradation (PMPD) may provide an opportunity for improved N utilisation in pastoral systems. We utilised *in vitro* and *in situ* forage incubations over two seasons to monitor PMPD and total protein degradation; and identify genetic and extrinsic sources of variation for PMPD within and among tall fescue, perennial ryegrass, white clover, and red clover elite varieties. *In vitro* tests indicate PMPD varies among forage species; and that variation in PMPD among perennial ryegrass varieties is influenced by genetic and environmental factors including temperature and N status. A further *in vitro* experiment identified 15% differences in total N loss via PMPD among individuals of the same variety, suggesting there is scope for genetic characterisation of the trait. Comparative analysis of porous and impermeable bags *in situ* indicate PMPD is the primary cause of protein loss in segmented fresh grass and legume leaf blades over the first four hours. Rumen models indicate a 10% decrease in PMPD causes a 3 to 6% increase in the flow of amino acids to the gut across a metabolisable energy (ME) range of 10.5 to 12.5 megajules ME/kg dry matter. These data suggest opportunities may exist to enhance protein utilisation in the dairy cow by improvements to PMPD. Realisation of these opportunities will require refinement of forage breeding practices.

P08: Selection for high molecular marker diversity to improve agronomic performance of *Lolium perenne* synthetics

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Agronomic performance of *Lolium perenne* synthetics was studied at three sites over two seeding years, each followed by two main harvest years (H1 and H2). Genetically narrow and wide polycrosses with 6 clones each were constructed based on Euclidean distances (E^2), using 184 polymorphic AFLP markers. In a first approach, 30 to 34 clones were assigned to three maturity groups, and polycrosses with the narrowest and widest possible combinations of 6 clones for each group were formed. Average E^2 was 39.2 for the narrow and 51.7 (+32%) for the wide polycrosses. In a second approach, 18 best performing clones were selected from the intermediate group and then arranged in three narrow (E^2 42.4) and three wide (E^2 53.6, +26%) 6 clone combinations. Seed was increased to syn-1 and syn-2 to establish plot trials.

The first approach resulted in a higher forage dry matter yield of the wide synthetics. The positive effect of a greater molecular diversity depended on the extent of the difference in E^2 but was sufficiently consistent over maturity groups, locations and years to result in an overall significant effect of +1.7% in H1 and +2.1% in H2. Wide synthetics were also scored significantly better for resistance to leaf spot diseases and vigour. Conversely, the second approach did not result in any significant effect on yield, and only vigour scores in H1 were influenced positively by a greater molecular diversity.

The results support the hypothesis that selection for high molecular diversity can improve agronomic performance of grass synthetics, but the difference in diversity must be large to obtain statistically significant results.

P09: SNP Genotyping for Axillary Tiller Formation in Perennial Ryegrass

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Tiller formation in perennial ryegrass (*Lolium perenne* L.) is of primer importance both for forage and turf grasses. Ryegrass with reduced number of tillers has beneficial nutritional values and superior lawn quality. In order to study genetic control of tiller formation a synthetic ryegrass population was created by intercrossing five genotypes within EU FP5 project 'GRASP'. Two rounds of selection (both positive and negative) for axillary tiller formation were performed. The mean number of axillary tillers per plant was 3.90 for plants from positive selection, 0.22 for plants from negative selection and 2.18 for population with no selection. Five ryegrass genes (*IAA1*, *RUB1 conjugating enzyme*, *BRI1*, *SHOOT1* and *TB1*) with putative functions in plant architecture and hormone response were chosen as candidate genes for genotyping. SNP genotyping in five ryegrass genes was done in all three populations. Three genes (*IAA1*, *RUB1 conjugating enzyme* and *BRI1*) out of five revealed significant deviations in allelic distributions across three populations under study. These results indicate possible role of the studied genes in ryegrass axillary tiller formation.

P10: Investigating Drought Tolerance in a Set of *Lolium perenne* Inbred Lines

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With climate change predicted to lead to warm and dryer summers in the British Isles a goal of our group is to develop tools to assist the breeding programme develop superior varieties with respect to drought tolerance. A set of *Lolium perenne* inbred lines have been characterized for their response to PEG induced drought in a hydroponics system. A Suppression Subtractive Hybridisation (SSH) approach has been employed to elucidate the genetic basis of the contrasting drought response. Leaf and root tissue was taken from two inbred lines with a contrasting response to the PEG induced drought stress. Subtracted libraries were then generated to identify genes under differential expression between the two lines in each tissue. Subsequently, a macroarray approach was used to monitor the expression profiles of selected candidate genes at various points in the drought regime. This allowed the identification of a number of genes whose expression profile is indicative of them having a role to play in responding to a PEG induced water stress.

P11: Evaluation of the agronomic value of *Lolium perenne* ecotypes and festulolium lines

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Perennial ryegrass (*Lolium perenne*) is a one of the most important cool-season species in many countries. However commonly used cultivars are still not very resistant to biotic and abiotic stresses. Therefore new germplasm are needed for breeding of new cultivars and for improving those which are already used. Meadow fescue (*Festuca pratensis*) is relatively resistant for biotic stresses like rust and snow mould diseases, but is less desirable than *L. perenne*. It has lower annual yield and poorer quality herbage. Hybrids of *L. perenne* and *F. pratensis* are intermediate between their parents in most characteristics and have a high potential as a breeding material.

Agronomic value of 12 Polish ecotypes and 18 introgression lines were investigated during three years. During the second year of the experiment green and dry mass production of 4 ecotypes and 5 festulolium lines was higher or on the level of Polish control variety Argona (green and dry mass production: 12,10 dt/ha and 4,10 dt/ha, respectively). During the third year of the experiment green and dry mass production of 6 ecotypes and 6 festulolium lines was higher or on the level of Argona (green and dry mass production: 16,20 dt/ha and 5,5 dt/ha, respectively). Those ecotypes and lines will be included into breeding programmes.

P12: Study of correlations by groups of characters for seed production in birdsfoot trefoil (Lotus corniculatus L.)

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As for seed production it was established that it is influenced by some characteristics which have a positive or negative influence. This, the direct correlations between seed yield, and the following characters is distinguished: number of inflorescences, number of pods, number of seed/pod, rate of seed setting. Dehiscence of the pods acts indirectly on the seed yield. Though the seed yield of birdsfoot trefoil is very much negatively influenced by the high degree of dehiscence in the pods, the studies pointed out an indirect, significant correlation between the dehiscence and the erect position of the shoots. As in majority of studied cases the sense and intensity of the correlations were similar for two seeding years it could by concluded that the relationships between the characters studied are genetically conditioned.

P13: Increasing Biomass for Bioenergy in Perennial Grasses

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Dedicated energy crops such as *Miscanthus x giganteus* are becoming extensively planted in Europe. *M. giganteus* is a sterile triploid arising through natural hybridisation of a tetraploid *M. sacchariflorus* and a diploid *M. sinensis*. *M. giganteus* displays a good balance of traits from each parent, combining rapid growth with a tolerance to low temperatures, making it an ideal biomass crop for Northern Europe.

Understanding of the genetic control of biomass performance traits is required to identify markers in order to accelerate breeding efforts and increase land use efficiency. *Miscanthus* takes three years to reach maturity in the field and so could benefit from marker assisted breeding. Target traits include the control of flowering time and plant architecture. The transition to flowering influences biomass yield as it determines the length of the growing season: delayed flowering generates increased yields. This additionally affects quality as flowering triggers senescence and nutrient reallocation from the aerial parts to the underground rhizome. Plant architecture consists of stem height, number and diameter, all of which are under genetic control and can be optimised for increased biomass yield. The ratio of stem to leaf may also affect biomass quality as the components of these organs are different. The UK *Miscanthus* breeding programme is based at IGER, and research tools are being developed to enable the association of phenotype to genotype.

P14: Phenotypic and molecular characterization of genetic resources of Nordic timothy (*Phleum pratense* L.)

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Timothy is the most important forage grass species in the northern parts of the Nordic countries. Serious winter damages, which significantly lower persistency and yields, occur regularly. The Nordic plant breeders have so far been quite successful in developing new, high-yielding varieties. However, the challenges today are to further improve winter hardiness and quality, especially in view of changing climatic conditions, as well as to produce varieties that might be exported to new markets. The Nordic Gene Bank (NGB) has a large collection of timothy accessions. However, neither the genetic structure nor the value of the collection for plant breeding purposes has been studied based on modern molecular tools. For plant breeding such information would make the collection more accessible and make it possible to improve specific traits more targeted and develop new varieties more efficiently. In this project, field trials in three Nordic countries and application of DNA marker techniques will be used to study the phenotypic and genetic variation of Nordic timothy germplasm in terms of distribution, dispersion history and important adaptive traits such as vernalization response and frost tolerance. The bio-geographical history of Nordic timothy will be studied using exotic germplasm, and the genetic basis of breeding materials broadened by identification of heterotic groups and new sources for improving important traits. The resulting data will be utilized both for improving genetic resource management of timothy and for breeding purposes.

P15: Analysis of the water soluble carbohydrate content in an unselected breeding pool of perennial ryegrass

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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.
During 2004, 2005 and 2006 we systematically determined by NIRS the WSC content of each forage cut in the year after sowing of the progeny test plots of our perennial ryegrass breeding program. These plots included diploid and tetraploid families, synthetics, varieties and were partly fertilized at two nitrogen application levels.
The variation of the WSC content among families is described. We calculated the correlations between the WSC content of the single cuts and the WSC content on an annual basis. Also correlations between the WSC content and dry matter yield, disease resistance, digestibility and nitrogen content and the effect of nitrogen fertilization are shown.

P16: Occurrence and characterization of *Neotyphodium* endophytes in Bulgarian Populations of *Lolium perenne*

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During a collection trip to Bulgaria 62 accessions of *Lolium perenne* were collected from 55 sites representing different lowland and mountain habitat types. Endophytes were detected in 59 accessions by microscopic analysis of seed. Infection level varied from >5 …100% with 50 out of 59 accessions showing infection levels higher than 50%. Endophyte-infected seeds were found more frequently on dry habitats than on wet sites, and more often at altitudes between 400-800m than at other altitudes. The morphological diversity of the endophyte isolates appeared to be greater than described originally for *Neotyphodium lolii*. Radial growth of many isolates was very slow (< 0.2mm/day) but faster growing isolates were also found. Isolates were assigned to 6 groups according to the morphological characteristics of the colonies grown on PDA. Detection of alkaloid levels revelaed that isolates were highly variable according their levels of lolitrem B, ergovaline, and peramine. One group of slow growing isolates did not produce ergovaline but high levels of lolitrem B. Clusters of isolates according their origin reflect the adaptation of endophyte/grass associations to specific climatic and geographic conditions.

P17: Selecting Genes in *Lolium* x *Festuca* Hybrids for Root Growth to Improve Soil Hydrology

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Plant roots can change the macro-porosity and hydraulic properties of soil by affecting soil structure and/or water repellence. It is considered feasible to design grassland systems to reduce runoff and lessen the impact of diffuse pollution on water catchments. Little is known about how different grasses affect soil profile hydraulic responses during either water excess or shortage. *Festuca* species have deeper rooting and water extraction characteristics than *Lolium* and this contributes to their greater drought resistance. Effects of rooting traits are being investigated at different scales using ryegrass, fescue, *Festulolium* hybrids and ryegrass genotypes with introgressed fescue genes. These include capabilities for penetrating hard compacted soils. It is postulated that deeper, stronger roots from *Festuca* species will increase soil porosity and the hydraulic conductivity of soil at both plot and headwater catchment scales. Drought-resistance traits have been transferred from two *Festuca* species onto *Lolium* chromosome 3, which is highly orthologous with rice chromosome 1 and implicated strongly in drought resistance and effective soil penetration traits. Primers designed from rice chromosome 1 are being used to map markers to the introgressed *Festuca* chromosome segments. These will be screened on a *F. pratensis* BAC library, with selected BACs sequenced and compared to rice to identify putative genes for rooting traits for improved soil hydrology.

P18: The Evaluation of Morphological Variability in Interspecific Hybrids *Trifolium pratense* x *T. medium* and Comparison with The Parental Species

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Trifolium pratense is high yielded as well as high quality fodder crop. However, it shows lower persistency, which could be overcome by hybridisation with a species creating rhizomes. Hybrids between *T. pratense* and *T. medium* have been previously obtained by embryo rescue and the number of chromosomes was evaluated by flow cytometry. This work was aimed at the evaluation of twelve morphological traits (stem weight/length, internodes numbers, length/width of a central leaflet of the triple leaf under the top head, length/ width of the central leaflet of the triple leaf on the 4th internodes, stem thickness on the 4th internodes, stem and head number per plants, intensity of white marks and average leaf area) in ten populations (550 plants) derived from F_1 hybrids and in the parental species. The significance of morphological differences was determined by ANOVA test. Nearly all examined traits were intermediate in hybrids; they reached higher values than in *T. medium* and lower values than in *T. pratense*. The stem number was significantly higher in ten hybrid populations compared with both parental species.

The financial support of the Ministry of Agriculture of the Czech Republic (grant no. G146034) is acknowledged.

P19: Utilizing Androgenesis in Poa Breeding

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The generation of haploids is a relatively infrequent event in most plant species. The derivation of paternal haploids via androgenesis has traditionally been used for the generation of inbred lines, transfer of cytoplasm and genome reduction in polyploidy species. This study investigates the use of a traditional breeding approach that takes advantage of the occurrence of androgenesis in *Poa* and the subsequent generation of dihaploid individuals. An interspecific hybridization program utilizing non-apotomic, *Poa arachnifera* (Texas bluegrass) as the female parent in crosses with apomictic *P. pratensis (Kentucky bluegrass),* apomictic *P. secunda (*Big bluegrass) and non-apomictic *P. ligularis* (Argentine bluegrass) has identified the generation of dihaploid individuals. Methods and the approach where we have characterized the frequency of this event via traditional and a molecular marker approach utilizing a series of *Poa* ssp. Molecular markers will be discussed. The value of using androgenesis in *Poa* breeding complemented with the use of molecular markers will be presented.

P20: 2D Electrophoresis Protein Expression Patterns of Perennial Ryegrass Inbred Lines and their F1 Hybrid

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Proteomics has developed into an important experimental approach for the investigation of complex cellular processes and network functions. Heterosis is one of the most widely used phenomena in plant breeding, but among the least understood in its biological and genetic mechanism and function. Proteomics could be a useful tool to further our understanding of the basis of heterosis. In this study abundant soluble proteins of two perennial ryegrass inbred lines and their heterotic F1 hybrid were analysed by 2D protein electrophoresis in 5 different overlapping pH-ranges: 3-6, 4-7, 5.3-6.5, 6-11 and 3-10 at two harvest time points in 2006. The objective of this study was to create protein maps for each of the three plant lines and to identify similarly and differentially expressed proteins for further studies. Similar protein patterns were obtained for the two inbred lines. This was in contrast to the protein pattern of the F1 hybrid line. Quantitative differences in the number of detected spots were observed among the two harvesting time points, mainly within the pH-range of 5.3-6.5. Mass spectrometric analysis of protein spots will further complement this study.

P21: Heritability of seed yielding capacity in tetraploid red clover (Trifolium pratense L.)

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Tetraploid red clover varieties are often characterised by an unsatisfactory level of seed yield leading to high production costs. Investigation of seed yield components showed a highly significant and positive correlation between the number of flower heads per plant and seed yield. In the phenotypical recurrent family selection plants are harvested individually and only plants with sufficient seed yield per plant start the next cycle. To investigate the heritability of seed yielding capacity we applied a divergent selection. Five families of 5 high seed yielding plants were allowed to intercross in one field and five families of low seed yielding plants in another field. Seed is harvested per offspring family for two years. In the first year of investigation analysis of variance for the seed yield per offspring family of the high and low seed yielding plants showed a highly significant difference between the two polycrosses. The heritability was estimated as the report between the difference of the mean of the seed yields of the high and low seed yielding parents and the difference of the mean of the seed yields of their progenies. The heritability was very high and amounted to 0.95. This indicates that eliminating plants with insufficient seed yields for the next cycle of recurrent family selection will improve the seed yield capacity of tetraploid red clover.

P22: Forage Yields in Some Serbian Urban Populations of Large-Flowered Vetch (*Vicia grandiflora* Scop.)

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Large-flowered vetch (*Vicia grandiflora* Scop.) is one of the most widely distributed vetch species in Serbia. It is often found growing in the same wild populations with narrow-leafed vetch (*Vicia sativa* L. ssp. *nigra* (L.) Ehrh.) and hairy vetch (*Vicia villosa* Roth), germinating in early autumn, flowering in April and producing seed in May.

A small-plot trial was carried out in 2005 and 2006 at Rimski Šančevi Experimental Field of the Institute of Field and Vegetable Crops, comprising twelve wild populations of large-flowered vetch collected in various parts of the cities of Belgrade and Novi Sad. All populations were sown in early October, at a seeding rate of 150 viable seeds m^{-2}, and were cut at full flowering and when first pods were forming.

Number of plants surviving until cutting ranged from 55 in NS-8 to 132 in NS-1 and NS-6. The highest green forage yield was in NS-3 (35.5 t ha^{-1}), while the highest forage dry matter yield was in NS-5 (11.6 t ha^{-1}). The lowest yields were in BG-4, with 21.4 t ha^{-1} of green forage, and 4.7 t ha^{-1} of forage dry matter.

P23: Improving Herbage Nonstructural Carbohydrates in Alfalfa

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High herbage nonstructural carbohydrate (NSC) concentration improves ruminant digestion. Concentration of NSC is genetically variable and increases during the day. We evaluated two populations from one cycle of divergent selection for NSC in alfalfa (*Medicago sativa* L.) harvested in the morning (AM: 9h00) or the afternoon (PM: 15h00) under field conditions. Populations (NSC+ and NSC-) were obtained by intercrossing 10 genotypes selected for high or low NSC concentrations from 500 genotypes of AC Caribou. Populations were evaluated for NSC, crude protein (CP), ADF, NDF, and dry matter yield (DMY). The NSC concentration (soluble sugars + starch) was predicted using NIR spectroscopy. The NSC+ population had higher NSC (116 vs 99 g/kg DM), and lower NDF (321 vs 337 g/kg DM) and ADF (248 vs 262 g/kg DM) concentrations than the NSC- population. Herbage DMY and CP concentration were not affected by populations and time of harvest. The PM-harvested alfalfa had greater NSC (126 vs 87 g/kg DM) and lower NDF (313 vs 342 g/kg DM) and ADF (241 vs 268 g/kg DM) concentrations than AM-harvested alfalfa. The increase in NSC concentration with NSC+ population or PM harvest was mainly due to an increase in starch concentration. Alfalfa NSC concentration can be increased through breeding and harvest management.

P24: Genome size variation among native bermudagrass [Cynodon dactylon (L.) Pers.] genotypes sampled from Turkey

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Bermudagrass is distributed throughout Turkey. But, there is almost no information available on ploidy and genetic diversity within the germplasm pool. Ploidy variation is a major consideration in bermudagrass breeding programs since a series of ploidy levels from diploid to hexaploid was reported for the species. This study was carried out to assess genome size variations among the bermudagrass genotypes collected throughout Turkey and use the information to determine ploidy levels of genotypes. Plant samples were collected systematically based on appearance and geographic distance. Genome size of genotypes was determined via flowcytometer. Our preliminary analyses indicate that 2C nuclear DNA content of genotypes varies from 1.0 to 3.5 pg/2C. Known tetraploid cultivars such as Riveria, Blackjack, and Sahara had about 1.95 pg/2C nuclear DNA content. Some genotypes had about 1.0 pg/2C and 2.5 pg/2C nuclear DNA content pointing out that those genotypes are diploids and pentaploids, respectively. But major part of genotypes had about expected 2.0 pg/2C nuclear DNA content, indicating that they are tetraploids. Based on our preliminary results, there is a considerable level of ploidy variation among bermudagrass germplasm accessions collected throughout Turkey, part of the major center of diversity for species.

P25: Development and Mapping of Expressed Sequence Tags (ESTs) in *Lolium perenne*

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Several linkage maps, mainly based on anonymous markers are now available for *Lolium perenne*. The saturation of these maps with markers derived from expressed sequence would provide complementary information useful also for QTL-mapping. Therefore we initiated an study with the main aim to map candidate genesin *Lolium* spp.

In a first step, EST markers were developed on sequences derived from a cDNA library, constructed from leaf tissue. 48 sequences with interesting homologies to genes with known gene function and involved in traits such as digestibility, nitrogen use efficiency, and disease resistance were selected. Seventeen markers were polymorphic in the CLO-DvP reference population.

We also developed EST markers from candidate genes involved in self-incompatibility. Three sources of candidate genes were used (1) putative S and Z genes, (2) genes involved in the signalling cascade triggered by a SI response, both identified by the cDNA-AFLP technique, and (3) S thioredoxine and S receptor kinase homologues. These EST markers were mapped, in the ILGI population, where S and Z had previously been mapped and/or the CLO-DvP reference population.

Success rates, levels of polymorphism and mapping results will be discussed.

P26: Development and Testing of a SSR-based Protocol for the Analysis of Essential Derivation in Diploid *Lolium perenne* L.

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In 1996 the Forage Plants section of ASSINSEL launched a study on essential derivation in perennial ryegrass. In that study the ability of AFLP markers to provide a reliable and meaningful estimate of genetic conformity of different diploid perennial ryegrass varieties and breeding populations was investigated. For the set of test accessions analysed, the AFLP protocol accurately reproduced the same relationships as were evident from examining their morphology and both these results were consistent with the genetic relationships known to exist within the different test groups.

Although that study clearly demonstrated that DNA-markers are useful tools to estimate genetic conformity between perennial ryegrass varieties, some of the intrinsic properties of AFLP markers make them less suitable to automation and to transfer among laboratories than, for example, SSR markers. The International Seed Federation (ISF) therefore financed a new study that builds on the previous one, to estimate new threshold values and eventually using alternative statistical approaches that could be used as a trigger to alternative dispute resolutions in case of putative essential derivation in perennial ryegrass, based on the use of SSR markers. The use of individual plants and bulks of several sizes was tested. In this presentation we will summarise the results of this study.

P27: Study the effects of symbiotic Mycorrhizal fungi (*G.fasiculatum and G. etunicatum spp.*) on quality traits of alfalfa (*Medicago sativa* L.*).*

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In order to study the effects of symbiotic mycorrhizal fungi on some quality trials in alfalfa, an experiment was conducted in 2004 in Alborz Research Center, Karaj, Iran. A factorial experiment with 3 factors as: mycorrizal fungi(myc) with 2 levels (with and without fungi), alfalfa varieties(var)with 5 levels (Ghareh Yonjeh, Krisary, FAO, Bami and Australia) and irrigation method(ir)with 5 levels (20%, 40%, 60%, 80% and 100% of field capacity) were established. Soil samples were collected from root region of alfalfa fields (5-30 cm. depth). Soils were mixed and examined for existence and identification of symbiosis mycorrhizal fungi. It was divided into 2 parts. The first part was sterilized for two times in laboratory and the second one was used as control. Seeds were sterilization by Benomil and sown in large plastic pots containing of sterilized and non sterilized soil (substrate). Data were collected for morphological traits and Dry matted production. For quality measurement, a sample of forage were dried and ground. The quality traits as crude protein (CP), dry matter digestibility (DMD), water soluble carbohydrates (WSC), total ash, crude fiber (CF), acid detergent fiber (ADF) and neutral detergent fiber (NDF) were measured using NIR technology. Data were analyzed for all traits. The results showed that the effects of symbiotic mycorrizal fungi (*G.fasiculatum and G.etunicatum)* were positively significant ($P<0.01$) on DMD and WSC. Interactions between (var×myc) and (ir×myc) were significantly varied on Wsc, ASH&ADF and DMD, ASH traits respectively.

P28: Agro ecologic system for white trefoil seed production (*Trifolium repens L.*)

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The conventional technology of seed production in white trefoil relies upon trefoil seeding in pure crop and on the application of specific herbicides against weeds. The researches presented in this scientific work present the possibility of achieving efficient seed yields by seeding white trefoil in associated crop (with *Dactylis glomerata* or *Festuca pratensis*), with no herbicide application, and the first or the second forage biomass harvest is used for animal grazing.

P29: Influence of Habitat on Genetic Diversity of *F. pratensis* Huds. and *L. multiflorum* Lam. Ecotype Populations from Permanent Grassland

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Permanent pastures and meadows harbour genetic resources of many forage plant species. Although these resources are often used for broadening gene pools in breeding programs, little is known about the importance of such sites for the conservation of genetic diversity. In order to evaluate genetic diversity present in permanent grassland and to identify valuable sites for conservation strategies, 12 ecotype populations collected at different habitats and four reference cultivars each of *F. pratensis* and *L. multiflorum* were analysed by means of SSR markers. Within population variation was lower for *F. pratensis* (92.6 %) than for *L. multiflorum* (97.1 %) as revealed by AMOVA. *F. pratensis* populations showed four main clusters. Ecotype populations were clearly separated from cultivars and formed three groups according to geographic regions; ecotype populations from the Swiss Alps, from eastern and from western Switzerland. Genetic diversity within populations was comparable for populations within each species, but within population diversity was lower for *F. pratensis* than for widespread *L. multiflorum.* The results suggest the existence of a single gene pool for *L. multiflorum* since no population differentiation could be observed. In contrast, *F. pratensis* populations were structured according to management and environmental factors prevailing at collection sites. However, it was not possible to identify specific habitat types as being particularly valuable for targeted conservation strategies.

P30: New Genomic Resources for Orchardgrass

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Topic Area: Applied Genomics

One of the initial requirements of utilizing genomic approaches in plant improvement is the availability of DNA sequence information. Toward the goal of generating sequence information for forage and pasture grasses, we are developing an EST library from orchardgrass, or cocksfoot (*Dactylis glomerata*). Tissues collected from orchardgrass included water and salt stressed shoot and roots, etiolated seedlings, and low temperature acclimated crowns. Library construction, sequencing, and bioinformatics are carried out through cooperation with the University of Illinois W.M. Keck Center for Comparative and Functional Genomics. The resulting EST library will be normalized, tagged for identification by tissue, and sequenced from both the 5' and 3' ends in order to include the 3'untranslated regions (UTR). SSRs identified from this library containing contigs/singletons will be aligned to rice chromosomes to determine predicted locations of the SSR markers. PCR primers designed from 3'UTR regions from other species contained high degrees of polymorphism and the same is expected from this orchardgrass library. Additionally, the sequence data from the library will be used to identify SNPs. The USDA ARS Forage and Range Research Laboratory has an extensive orchardgrass improvement program and our objective is to identify phenotypic associations and apply the markers to a marker-assisted selection program for increased salt tolerance and winter hardiness. Sequence information and resulting primers will be presented.

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P31: Techniques for Marker-free Transgenic Alfalfa

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Selectable marker genes (SMGs) are often linked to useful genes to efficiently produce transgenic plants, but are not generally useful outside the lab. Techniques to avoid the presence of SMGs in transgenic plants are available, but have not yet been implemented in forage crops. We are assessing the efficiency of co-transformation and markerless transformation in obtaining marker-free transgenic alfalfa. Co-transformation with two T-DNAs, each carried by a different culture of *Agrobacterium tumefaciens*, was performed. Fourteen putative co-transformed plants were regenerated from 400 leaf explants in two experiments. Southern and realtime qPCR analyses have indicated integration of one or few copies of each T-DNA in most of these plants. Some plants were crossed to a non transgenic plant, and two progenies examined for transmission of both T-DNAs using PCR. Segregation of the two T-DNAs was observed in one progeny, indicating the feasibility of this marker-free approach. We are now attempting to improve co-transformation efficiency. For markerless transformation, we are using both a SMG and a reporter gene (GUS) to estimate the percentage of transgenic somatic embryos that can be obtained without selection. In the first experiment, underway, 1.7% of the embryos were putatively transgenic. Further experiments are in progress and their results will be presented.

P32: Flexibility In Grasses Breeding Management With An Integrated Research Software

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DORIANE specialize in management of vegetal breeding research since 1984. Thanks to the contribution of breeding companies and research institutes, involved in a large range of crops and laboratory activities, the efficiency and the flexibility of the concept of "experiment cycle" is now recognized.

Objectives : software components and data base architecture able to manage complex "experiment cycles" as it is required by grasses breeding. The aims of these tools are the sharing of algorithm development, the genotype data base management, phenotype and molecular markers properties, and perennial management of protocols.

Materials : genotype management and analysis functions of LABKEY™-SELEC, LABKEY™-MOLECULAR-MARKERS and LABKEY™-EXPERIMENTATION specifically customized to manage grass parents collections, production of clone and polycross experiment, backcross based conversion, seed testing and trials, genotype profiling analysis combining phenotype and molecular markers information, ascending, descending and moderated genealogy, distances, dendrograms and PCA.

Method : writing of an original customizing of the LABKEY™ modules. The steps of genotype management are presented in experiments themselves following rules and data architecture stored in LABKEY™ protocols. This method for the presentation of grass breeding management permits to preserve the confidentiality of the methods actually in use in laboratories.

Result : a tool which flexibility gives ability to feet very accurately to needing of breeding activity, even in the very particular cases of grass breeding.

P33: False SNPs Arising from MassARRAY Genotyping: "The Paralogue Problem"

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We employed MassARRAY, a high throughput MALDI-TOF Mass Spectrometry based SNP genotyping assay, to study changes in allele frequencies in perennial ryegrass under selection for high and low frost tolerance. Un-expectedly high levels of SNP heterozygosity were observed in one of the frost tolerance associated candidate genes IRIP (Ice Recrystalization Inhibition Protein). We therefore validated the IRIP SNP genotyping results obtained from the MassARRAY assays by 1) sequencing and 2) a modified MassARRAY assay. Validation results suggested that elevated heterozygosity in the original MassARRAY results was an artefact of paralogous sequence variation, rather than real SNP variation. Further investigations support that the perennial ryegrass IRIP gene is part of a gene family consisting of at least two highly conserved paralogues. The "paralogue problem" of SNP genotyping is discussed further.

P34: Can We Document Genetic Yield Improvements in Annual Ryegrass (*Lolium multiflorum* L.)?

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Continuous yield improvements over the last 70-100 years have been documented for major agronomic species such as maize (Zea mays L.), wheat (*Triticum aestivum* L.) and soybean (*Glycine max* Merr.). Critical studies document that, in general, half the yield increase could be attributed to genetic improvement and the remainder through improved crop management. Annual ryegrass in the USA is sown to > 1.3 Million hectares annually, most of it over seeded as a winter-annual into permanent warm season pastures of the humid South. The semi-weedy characteristics of this species - rapid emergence and excellent seedling vigor – ensure rapid establishment and enable grazing as soon as 45 d post seeding. Most states in the southern USA conduct annual ryegrass cultivar evaluation trials at multiple locations. Cultivars Gulf and Marshall, both released in Mississippi, have been used as standard checks for the last 15-20 years. Trial results are a matter of public record and available through the websites of the State Agricultural Experiment Stations (SAES). We utilized trials records from Alabama, Georgia, Louisiana, and Mississippi to investigate genetic yield improvements over the last 20 years looking both at overall seasonal dry matter yields as well as yield distribution. Small improvements in forage yield can potentially have a large impact on total animal gain per ha. Results indicate that there has been no consistent yield improvement in annual ryegrass over the last two decades. In the presentation we will discuss possible reasons for this lack of progress.

P35: Inheritance of crown rust resistance in perennial ryegrass

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Genetic resistance in perennial ryegrass, *Lolium perenne* L., provides an effective means of controlling crown rust, caused by Puccinia coronata Corda f. sp. *lolii* Brown. Three perennial ryegrass plants were selected for a genetic study based on low disease reaction to different single pustule isolates of crown rust. Crosses were made to determine the mode of inheritance of the resistance in each plant. Each of the three resistant plants was crossed with a highly susceptible plant of the variety Aurora. The F1 populations were evaluated for resistance/susceptibility to 6 - 10 different single pustule isolates of crown rust using a detached leaf assay. In two of the three F1 populations the percentage of susceptible plants varied from 11 - 78 % depending on the rust isolate used. This suggests that in the corresponding parental genotypes, resistance to different *P. coronata* pathotypes is controlled by several genes. Evaluation of the third F1 population revealed that the resistance in this specific cross is controlled by one single dominant gene. Segregation ratios of 1:1 (susceptible : resistant) was observed in all of the six isolates tested. Moreover, the same plants were resistant or susceptible, respectively, to all of the six isolates tested. This material offers great opportunities for molecular studies. Selected plants of the F1 generation were again crossed with a susceptible Aurora plant to produce F2 seeds. Evaluation of the F2 populations is now underway.

P36: Cytogenetic Diversity as a Source of Genetic Variation in Aegilops Species and Populations in Iran

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Variations were observed in ploidy level, chromosome pairing, chiasma frequency and distribution as well as chromosome segregation were analysed in several populations of *Aegilops* species namely: *A. triuncialis*, *A. umbellulata*, *A. tauschii* and *A. crassa* and *A. speltoides*. The populations of *A. tauschii*, *A. triuncialis*, *A. umbellulata* and *A. speltoides* possessed 2n = 14 chromosomes (diploid), while populations of *A. crassa* possessed n = 28 and 42 (tetraploid & hexaploid). Tetraploid species showed diplontic behaviour and formed bivalents only while diploid species of *A. speltoides* showed the occurrence of quadrivalents and hexavalents possibly due to the outbreeding nature of this species and structural heterozygosity. Cluster analysis of meiotic data showed distinctness of the *Aegilops* species in their meiotic behavior, although significant variations were observed in the chiasma frequency and distribution among different populations of each species. Twenty- seven populations of 6 *Aegilops* species were screened for the presence of B-chromosomes. Statistically significant effect of B chromosomes on chiasma frequency and distribution was found in some of the species, hower details of these effects varied in different populations. Unreduced gamete formation was noticed in some which may be of practical use in breeding of aegilops. Cytomoxis and meiotic abnormalities formed such unreduced gametes.

P37: Investigation of Some Wild Annual VETCH *(VICIA* L)

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Investigation of annual vetch (*Vicia villosa* Roth., *V. angustifolia* L., *V. hirsuta* Gray) cenopopulations has been carried out since 1998. Genefund is being collected, morphobiochemical characteristics and adaptive potential are being studied. Plant mass, stem, leaf and inflorescence ratio in the mass and crude protein content have been measured. Plant growth phenological stages are recorded. Diseases and damage done have been described.

Experiments have shown that by preserving the characteristics specific to the cenopopulations the tested species *(Vicia villosa* Roth, *V. angustifolia* L., *V. hirsuta* Gray) readily adapt to different years' diverse ecological conditions. *V. villosa* was distinguished for the largest biodiversity. Averaged data suggest that according to crude protein content in plant aboveground mass, stem, leaf and inflorescence ratio in plant mass all the tested spontaneous species were more valuable than the cultivated *V. sativa* species. For detailed tests and application in plant breeding cenopopulations were selected according to earliness of flowering, productivity, crude protein content, and disease resistance.

P38: New Microsatellite Loci for Red Clover (Trifolium pratense L.) and Study its Polymorphism

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A microsatellite map of red clover was constructed until quite recently by Sato et al. (2005). Kölliker et al. (2006) defined other microsatellite loci. The objective of this study was obtaining new SSR markers to its other application for genetic diversity study eventually QTL analysis. Resources of SSR oligonucleotides was white clover (Trifolium repens). In last years was found in the same way 13 SSR markers of it 11 was polymorphic (Jungmannova, Repkova, 2006). Products was amplificated mainly in czech cultivars therefore markers were not suitable for genetic diversity evaluatin among cultivars of core collection. In the same way was found other 16 novel SSR markers. Allelic variability was assessed on 11 parents' genotypes.

P39: Expressed Sequence Tag - derived Microsatellite Markers of Perennial Ryegrass (*Lolium perenne* L.)

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Simple sequence repeat (SSR) markers are widely used for cultivar identification, variety protection, diversity studies, genetic linkage mapping and trait dissection in the key grassland species perennial ryegrass (*Lolium perenne* L.). An expressed sequence tag (EST) library of perennial ryegrass has been exploited as a resource for microsatellite marker development in order to increase the number of publicly available primer sequences and to ensure a reasonable genome coverage required for linkage mapping and trait dissection. EST-SSRs are of particular interest because they may be functionally associated with trait variation and are highly transferable to other pedigrees and species, which makes them valuable as anchor markers in genetic mapping studies. Out of 25.744 ryegrass EST sequences, 1.458 microsatellite motif containing ESTs were identified using the Perl script Micro SAtelitte, representing 955 non-redundant SSRs (Asp et al. 2007). Within a selection of 744 SSR motif containing ESTs, one to three primer combinations (1.221 in total) were developed using Primer 3 software. Primer amplification was tested within six *L. perenne* and two *L. multiflorum* genotypes, representing mapping (grand-) parents of four populations used for trait dissection. Out of a total of 1.221 primer pairs, 618 (50.6 %) clearly amplified products, representing 468 different ESTs (62,9 % of the 744 unique ESTs). One primer combination per EST amplifying one or two bands of expected size was used to screen polymorphisms within the mapping population VrnA (Jensen et al. 2005). Within a subset of eight VrnA F2 genotypes, 310 SSRs were successfully amplified, including 144 polymorphic SSR markers.

P40: Nuclear DNA content and ploidy determination of *Dactylis* germplasm accessions using flow cytometer

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The grass genus *Dactylis* L. is a polyploidy complex which consists of over twenty mainly diploids ($2x = 14$) and tetraploids ($4x = 28$), although the presence of hexaploids has also been reported ($2n = 42$). Accurate estimations of nuclear DNA contents are important for mapping of plant genomes and development of strategies for isolation of important plant genes. The importance of nuclear DNA content, as useful taxonomic markers has been also stressed in many studies based on different groups. However, nuclear DNA content informations are available only for tetraploid *Dactylis glomerata* L. within the genus and they vary between 6.4 pg/2C and 12.4 pg/2C. The objectives of this study were to assess nuclear DNA content of more than 100 accessions, obtained from gene banks throughout the world representing 20 different taxon of genus *Dactylis* L. and correlate nuclear DNA content information with ploidy levels of the accession**.** Based on the results of our study, there is a considerable level of nuclear DNA content variation within the genus as it varies from 3.65 pg/2C to 5.12 pg/2C and from 7.47 pg/2C to 9.11 pg/2C in diploid and tetraploid taxons, respectively. No hexaploids were found in the present study. Genome structure, phylogeny and evolution of the grass genus *Dactylis* will be also discussed based on the nuclear DNA content characterized in the study.

P41: Seed Germination and Seedling Growth of Three Vetch Cultivars under Moisture Stress

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The aim of this study was (i) to investigate the response of three vetch local cultivars large seeds, medium seeds and small seeds to osmotic stress (-0.33, -0.66, -0.99 and -1.72 Mpa) during germination and seedling growth, (ii) to identify characters that can be used for screening genotypes, (iii) to determine the effects of cultivars on yield and yield components of rainfed vetch in arid (150 mm rainfall) and semiarid (377 mm) regions. Large seed cultivars had higher percentage germination and germination speed under moisture stress than small seed cultivars, but germination speed was more sensitive to change the osmotic potential than percentage germination. Root and shoot weights of all cultivars were reduced when osmotic potential was decreased, but the extent of reduction in root growth was less than that for shoots. Vetch plants at the semi-arid location (Jubieha) had higher seed yield (kg ha^{-1}), 1000 seed weight (g) and plant height (cm), than those grown at the arid location (JUST). Vetch plants from larger seeds cultivar had higher seed yield, 1000 seed weight and plant height than those from smaller seeds cultivars.

P42: Improvement of the quality of grass-clover mixtures for forage production

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Traits such as protein stability, water-soluble carbohydrates content and fatty acid compositions are determining factors for forage quality. They influence the intake and the profit of animals, the composition of urine, the composition of milk and meat and finally the consumer's health. The last years, the demand for clover and grass/clover mixture for forage production has risen. Besides the fixation of nitrogen, the association of clovers with grasses in the fodder presents several additional advantages. As it is the case for protein, clovers would have a protective effect on the grass fats during wilting, ensiling and possibly in the rumen. Specific enzymes and metabolites of the clovers would inhibit the grass lipases. Our new project, started in 2007, aims to acquire knowledge and techniques to allow the breeding of grasses and clovers for important nutritional aspects, namely the protein stability, fatty acid composition and enzyme activity (polyphenoloxydase, lipase) and metabolites (saponines, polyphenol) interacting with protein and fat breakdown. The first objective of the project is to develop screening techniques for the evaluation of these different quality aspects on large numbers of plants. NIRS, gas chromatography and chemical analysis are used and compared. The second objective is to evaluate the existing variation and the heritability of the traits within breeding populations and between varieties using the previously developed methods. The last objective is to evaluate the effect of wilting and ensiling on the fodder quality of grass-clover in relation with the protective effect of clover on unsaturated fatty acids in an *in silo* and *in vitro* study.

P43: Effect of cutting schedule on red clover seed production

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Because of the physiological self-sterility and flower anatomy, the red clover (*Trifolium pratense* L.) is pronouncedly cross pollinated plant species. Pollinating insects must be present to ensure a successful seed production. Besides that, some seed yield components are particularly important for successful seed production.

Cutting date is used for timing of the beginning and duration of flowering period in seed crops, with the intention of synchronizing flowering period with maximum activity of pollinating insects. The effect of cutting schedule on red clover seed yield and yield components was studied in the second year of two cycles of cultivation (2005, 2006). Four cutting schedules with variable dates of initial cutting were tested. First growth was used for seed production in the c_1 schedule and second growth in the c_2-c_4 schedules (c_2-budding, c_3-start of flowering, c_4-full flowering). Domestic red clover cultivar- Kolubara was tested for: number of productive stems per unit area, number of inflorescences per unit area, number of flowers per inflorescence, number of seeds per inflorescence and seed yield. The earliest cut of hay (in the c_2 schedule) achieved the best balance among the seed yield components during the second growth, using for seed production in both of years.

P44: Complete evaluation of the Czech core collection of *Trifolium pratense*, including morphological, molecular and phytopathological data

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During the year 2006 the Czech national core collection of the red clover (*Trifolium pratense* L.) was established basing on morphological data obtained on the set of 57 tetraploid accessions (varieties and newly bred varieties) and 130 diploid accessions (varieties, newly bred varieties and wild forms collected in the nature) of the world collection. The core collection includes 76 origins, which were evaluated in detail. New molecular and phytopathological data were obtained during the year 2007. For study the genetic diversity were used microsatellites, highly informative molecular markers. The whole core collection (76 origins) had been evaluated from the point of view of resistance to important fungal and viral pathogens. Tested plants had been inoculated by spore-suspension of *Fusarium* spp. fungi and also by BYMV inoculum. After cultivation time the evaluation was introduced. The obtained data had been added to complete the core collection evaluation. All the obtained data and evaluated characters were used in construction of data matrix and then they were included into the cluster analysis. The analysis was performed in the software Statistica for Windows for all the 76 accessions together. Complete linkage method was used for clustering and Euclidean distance as the measure of distance. Most important results of the analysis are presented and main clusters within the core collection are compared and described.

P45: Genetic improvement of traits conferring enhanced economic and environmental sustainability to grassland agriculture

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Increased grass nutrient use efficiency, reduced fertiliser use, lower dietary intakes of nitrogen and improved nitrogen conversion in the rumen through use of high sugar grasses and/or those with reduced proteolysis will reduce nitrogen leaching to the environment and provide increased profit margin to farmers. To improve performance of these traits in elite grass varieties, use is being made of genetic mapping and marker assisted selection and breeding methods. Perennial ryegrass genotypes have been identified that are suitable for mapping new sources of genetic variation that currently do not exist in elite breeding populations. To harness complementary traits from fescues (*Festuca*) species, a number of donors with new combination of genes in elite ryegrass genetic backgrounds have also been made available. Crosses have been made in such a way that they identify new genes that are not present in elite breeding population or cultivars. Availabilities of such crosses are allowing us simultaneous mapping as well as fast-track incorporation of such variations into elite ryegrass cultivars. To achieve real progress in variety development using marker assisted selection, a number of steps, as followed in practical breeding, are being taken up both in the development and evaluation of segregating populations, and in accumulation of useful QTL alleles in elite grass varieties.

Preference for presentation - Oral

P46: Identification and characterization of genes involved in self-incompatibility (SI) in *Lolium perenne*

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Self-incompatibility (SI) prevents plants from self-fertilization, hence the efficient production of inbred lines and hybrids. SI in *Lolium perenne* is controlled gametophytically by two multiallelic and independent loci, *S* and *Z*, which have been mapped to linkage groups 1 and 2 respectively. None of the gene products of *S* and *Z* have yet been identified. Six suppression subtracted cDNA libraries were developed from in-vitro pollinated stigma subtracted with unpollinated stigma to identify SI related genes. A total of 2112 clones were generated and after differential screening 768 clones were sequenced. Previous comparative mapping work had identified regions on rice chromosomes 5 and 4 with synteny of *L.perenne S* and *Z* loci, respectively. Through a BLAST search against rice sequences, 82 out of a total 239 rice homologies are on rice chromosomes 4 and 5, the rest on other rice chromosomes and are potentially involved in the downstream SI responses, including protein kinases, signal transduction genes, protein regulation genes and genes with unknown function. The candidate genes expression pattern was tested on a pollination time-series by RT-PCR and some showed a tissue specific expression pattern, only in pollinated stigma, implying their roles in *L.perenne* SI response.

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