Pre-breeding - fishing in the gene pool

## **Book of abstracts**



European Plant Genetic Resources Conference 2013

# EUCARPIA

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

Section Genetic Resources

## Pre-breeding - fishing in the gene pool

European Plant Genetic Resources Conference 2013

Abstracts of oral presentations and posters





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## **Organising Committee**

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Acknowledgement to the City Council of Malmö for hosting the welcome party.

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## Foreword

This Book of Abstracts "Pre-breeding – fishing in the gene pool" includes the summary for each of the invited keynotes, volunteer talks and posters presented at the EUCARPIA Genetic Resources Section Meeting held at the campus of the Swedish University of Agricultural Sciences (SLU) in Alnarp from 10 to 13 June 2013. This meeting gathers various members of the gene bank community of practice as well as users of the genetic resources from all around Europe and the World.

The selected overall theme for the meeting is "Pre-Breeding - fishing in the gene pool" because we want to discuss how we better can use our genetic resources to cope with the issues being faced today by humankind. Germplasm enhancement or pre-breeding refers to the transfer or introgression of genes and gene combinations from non-adapted sources into breeding materials. It should therefore be understood as an early component of sustainable plant breeding that deals with identifying a useful character, capturing its genetic diversity, and putting genes into usable forms that will be further used in population improvement or line development.

During this gathering we will address the role of genomics for adding value to genebank holdings and facilitating their management, the conservation and use of crop wild relatives in germplasm enhancement, how plant genetic resources serve multifunctional agriculture and what traits gene banks can offer to address climate change. We will also learn more about the role of gene banks for ensuring food and feed, and how genetic diversity contributes to human health and nutrition. Last but not least we will assess how genetic endowments can be used in crop breeding to reduce agriculture's footprints, as well as on the management of intellectual property for plant genetic resources, particularly in the light of increasing public-private partnerships on pre-breeding. The book content follows the order indicated in the program of this conference

The hosts of this meeting are Nordic Genetic Resource Center (NordGen) and the SLU, which have been engaged in collecting, conserving, characterizing, evaluating, documenting and facilitating the use of genetic resources for agriculture since their inception. We hope that during your visit you will enjoy the tours to both of them as well as the surrounding environments, where you can find thousands of trees, bushes and flowers, together with landscape and natural sciences laboratories, glasshouses, the biotron, the rehabilitation garden and a range of beautiful buildings. The 150-years old Alnarp castle dominates the center of the park and is a clear icon of this campus due to its beautiful architecture.

We welcome the participants to the EUCARPIA Genetic Resources Section Meeting 2013 and wish you a pleasant stay in southern Sweden while actively engaging in exchange of views during this agro-biodiversity event here in Alnarp.

Alnarp, June 2013

#### Arni Bragason

Director, NordGen Chairman, Steering Committee Rodomiro Ortiz

Professor, Genetics & Plant Breeding, SLU Chairman, Scientific Committee

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## Program for Genetic Resources section meeting 2013 Pre-breeding – fishing in the gene pool

## June 10: Arrival Day 0, evening

19:00-20:00 Welcome Party, City Hall, Stortorget in Malmö

### June 11

## Day 1, morning

- 08:15 Busses leaves from Gustav Adolf Torg and Comfort hotel
- 08:30 Registration At venue, Alnarpsgården, Sundsvägen 6, Alnarp
- 09:15 Welcome and short introduction by Arni Bragason, NordGen, Sweden
- 09:20 Eva Thörn, EUCARPIA, Plant Genetic Resource Section, Sweden
- 09:25 Jose Vouillamoz, EUCARPIA, Switzerland

#### Session 1: Genomics of genebanks

Chair: Anders Carlsson, Swedish University of Agricultural Sciences, Sweden

- 09:30 Nilsson-Ehle KEYNOTE "Genome wide association studies (GWAS) help develop blueprint for better utilizing natural variation in rice" funded by det Kongelige Fysiografiska Sällskapet i Lund by **Susan McCouch**, Cornell University, US
- 10:00 Genebanking in the sequencing era **Robbert van Treuren**, Wageningen University and Research Centre (WUR), The Netherlands
- 10:20 Adaptation of oilseed rape, barley and Brassica rapa to the future climate and increased [CO2] **Rikke Bagger Jørgensen**, Technical University of Denmark (DTU), Denmark
- 10:40 Coffee break, 1<sup>st</sup> floor at the venue place

#### Session 2: Food Security

Chair: Isaak Rashal, University of Latvia, Latvia

11:00 KEYNOTE "Genebanks and food security" by **Ruaraidh Sackville Hamilton**, International Rice Research Institute (IRRI), The Philippines

11:30 Seeds of Discovery (SeeD): An initiative to systematically explore and mobilize novel genetic variation into maize and wheat breeding programs - **Sara Hearne**, International Maize and Wheat Improvement Center (CIMMYT), Mexico



- 11:50 Exploiting the INRA breadwheat collection to sample accessions used in French BREEDWHEAT project - **Audrey Didier**, French National Institute for Agriculture Research, France
- 12:10 Enhancing Global Capacity for Pre-breeding Chikelu Mba, FAO, Italy
- 12:30 Lunch break, 1<sup>st</sup> floor at the venue place

## Day 1, afternoon

## Session 3: Nutrition and Health: Genetic diversity for health and nutrition

Chair: Külli Annamaa, Jogeva Plant Breeding Institute, Estonia

- 14:00 KEYNOTE "Genetic diversity for health and nutrition" by **Hilde Nybom**, Swedish University of Agriculture Sciences (SLU), Sweden
- 14:30 A large scale association study for aroma wheat components in bread wheat **Jihad Orabi**, University of Copenhagen (KU),
- 14:50 Genotypic variations in cadmium concentration of winter wheat grain an opportu nity for healthier bread - **Sonja Maric**, Josip Juraj Strossmayer University, Croatia
- 15:10 Coffee break, 1<sup>st</sup> floor at the venue place
- 15:45 PGR of oat for gluten-free diets **Igor Loskutov**, N.I. Vavilov Institute of Plant Industry, Russia
- 16:05 Italian grape genetic diversity evaluation and exploitation **Valeria Terzi**, Genomics Research Centre (CRA-GPG), Italy
- 16:25 Poster Introduction, Jette Nydam Hansen, NordGen, Sweden
- 17:50 Busses to Malmö Comfort hotel and Gustav Adolfs torg

### Day 1, evening, free time in Malmö

### June 12

## Day 2, morning

08:30 Busses leaves from Gustav Adolf Torg and Comfort hotel

## Session 4: Crop wild relatives, multifunctional agriculture, climate change

Chair: Johannes Engels, Bioversity International, Italy

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- 09:00 KEYNOTE "Wild relatives in prebreeding: Fishing the genepool with dynamite" by Hannes Dempewolf, Global Crop Diversity Trust, Germany
- 09:30 Extending use of the crop gene pool: developing national strategies for crop wild relative conservation and use **Nigel Maxted**, University of Birmingham, England, UK
- 09:50 Ecogeographical approaches to characterize CWR adaptive traits useful for crop adaptation **José M. Iriondo**, King Juan Carlos University, Spain
- 10:10 Coffee break, 1<sup>st</sup> floor at the venue place

Session 5: Genetic resources and traits to address climate change Chair: Åsmund Bjørnstad, Norwegian University of Life Sciences (UMB), Norway

- 10:40 KEYNOTE "Genetic resources and traits to address climate change" by **Andy Jarvis**, International Center for Tropical Agriculture (CIAT), Colombia
- 11:10 The Focused Identification of Germplasm Strategy (FIGS): An approach to pre-breeding for water-limited environments Hamid Khazaei, University of Helsinki, Finland
- 11:30 Gene hunting at the Carlsberg Laboratory employing historic barley mutants Mats Hansson, Carlsberg Laboratory, Denmark
- 11:50 Abiotic stress tolerance in 138 barley accessions tested in the future Nordic climate **Cathrine Ingvordsen**, Technical University of Denmark (DTU), Denmark
- 12:10 Lunch break, 1<sup>st</sup> floor at the venue place

#### Day 2, afternoon

- 13:15 Excursion (in 4 groups). Starting point outdoors at venue.
- 16:00 Busses to Malmö Comfort hotel and Gustav Adolfs torg

#### Day 2, evening

18:00 Malmö castle and conference dinner. Walking distance from hotels, ca 1 km.

### June 13

#### Day 3, morning

08:30 Busses leaves from Gustav Adolf Torg and Comfort hotel

#### Session 6: Genetic endowments for reducing agriculture's footprints Chair: Heiko Becker, The University of Göttingen, Germany

- 09:00 KEYNOTE "Leveraging ecotypic diversity to improve breeding programmes in the forage grass *Lolium perenne*" by **Matthew Hegarty,** Aberystwyth University, Wales, UK
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- 09:30 Nitrogen use efficiency indices in old and modern barley genotypes Ari Rajala, MTT Agrifood Research, Finland
- 09:50 Developing a European on farm conservation strategy for landraces Valeria Negri, Università Studi Perugia, Italy
- 10:10 Diversity in the perennial grasses species recommended for conventional and low input agriculture **Elzbieta Czembor**, Plant Breeding and Acclimatization Institute, Poland
- 10:30 Coffee break, 1<sup>st</sup> floor at the venue place

#### Session 7: Intellectual property management and genebanks Chair: Antonio Michele Stanca, Genomics Research Centre (CRA-GPG), Italy

- 10:50 KEYNOTE "Access to genetic resources and plant breeding the effects of IP and ABS obligations" by **Anke van den Hurk**, Plantum, The Netherlands
- 11:20 The new Genebank CGIAR Research Program **Denise E. Costich**, International Maize and wheat Improvement Center (CIMMYT), Mexico
- 11:40 The impact of international agreements on genebank management in Europe -Theo van Hintum, Wageningen University and Research Centre (WUR), The Netherlands
- 12:00 Plant Genetic Resources: Characterization and Utilization: a journal with a future **Robert Koebner**, CropGen International, England, UK
- 12:20 Lunch break, 1<sup>st</sup> floor at the venue place

#### Day 3, afternoon

- Chair: **Ulrike Lohwasser**, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany
- 13:30 Public Private Partnership in Pre-Breeding: Combining Knowledge from Field and from Laboratory for Pre-breeding in Barley **Ahmed Jahoor**, Nordic Seed, Denmark
- 13:50 The Nordic Public-Private Partnership (PPP) for pre-breeding in perennial ryegrass (Lolium perenne L.) Odd Arne Rognli, Norwegian University of Life Sciences (UMB), Norway
- 14:10 Prebreeding for Future Challenges in Nordic Apples **Dag Røen**, Graminor AS, Norway
- 14:30 Safety duplicating global ex-situ collections: Taxonomic and institutional representation in the Svalbard Global Seed Vault collection **Ola Westengen**, NordGen, Norway

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- 15:00 Closing words, **Håkan Schroeder**, SLU, Swedish University of Agricultural Sciences, Sweden
- 15:15 Coffee break, 1<sup>st</sup> floor at the venue place
- 16:15 Busses to Malmö Comfort hotel and Gustav Adolfs torg

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Jose Vouillamoz

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## **Oral Presentations**

(Ordered according to the program)

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1 Genomics of gene banks







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## Genome wide association studies (GWAS) help develop blueprint for better utilizing natural variation in rice

Susan McCouch<sup>1</sup>, Mark Wright<sup>1</sup>, Chih-Wei Tung<sup>1</sup>, Adam Famoso<sup>1,2</sup>, Joshua N. Cobb<sup>1,2</sup>, Sam Crowell<sup>1</sup>, Genevieve DeClerck<sup>1</sup>, Randy Clark<sup>2,3</sup>, Leon Kochian<sup>3</sup>, Anthony Greenberg<sup>4</sup>, Pavel Korniliev<sup>4</sup>, Francisco Agosto-Perez<sup>4</sup>, Liakat Ali<sup>5</sup>, Jason Mezey<sup>4</sup>, Anna McClung<sup>6</sup>, Georgia Eizenga<sup>6</sup>

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Understanding the relationship between genotypic and phenotypic variation lies at the heart of the study of genetics and is also critically important to applications in plant and animal breeding. Here we present a genome-wide association study (GWAS) based on genotyping a rice diversity panel with a high-density SNP array and systematically phenotyping the panel for a range of agronomic, physiological and morphological traits. The use of wild rice ancestors, ancient landrace varieties, and modern, elite cultivars, thanks to seeds conserved in global germplasm repositories, provide insight into a wide range of natural genetic variation that has been largely ignored in modern rice genetics and breeding. The advent of high throughput sequencing and genotyping technologies makes it possible to examine genome-wide patterns of variation and to detect sub-population structure within a species. In this study, we used GWAS to identify numerous common variants influencing complex traits in rice, and detected significant heterogeneity of genetic architecture across subpopulations and environments. This work establishes an open-source translational research platform for genome-wide association studies in rice that directly links molecular variation in genes and metabolic pathways with the germplasm resources needed to accelerate varietal development and crop improvement for diverse environments.

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## Genebanking in the sequencing era

Rob van Treuren & Theo van Hintum

Centre for Genetic Resources, the Netherlands (CGN) Wageningen University and Research Centre (WUR), P.O.Box 16, 6700AA Wageningen, The Netherlands. robbert.vantreuren@wur.nl

During the last decade, advances in sequencing technologies have made it possible to generate increasing numbers of data against decreasing costs. These developments have resulted in numerous sequencing projects, including the publication of the entire genome of several (crop) species. More and more samples from species with reference genomes are being re-sequenced to identify variation at genes, and to explore its relationship with useful phenotypic variation. Although the bioinformatics component and the lack of proper phenotyping are still limiting factors in genomics, such studies will eventually pave the way for unravelling the genetic basis of biological variation.

As genebanks generally harbour massive numbers of samples of a crop's gene pool, their collections are considered important reservoirs of variants for traits of interest. Therefore, the advances in sequencing technologies can be expected to have a large impact on genebanks. However, currently most genebanks manage a large variety of crops, have no or limited access to high-tech research facilities, and do not possess the necessary genomics and bioinformatics expertise. In addition, genebank budgets do generally not allow for the required investments. Therefore, to be able to anticipate the massive changes that can be expected, genebanks will need to start thinking about how their activities will change in the sequencing era.

It is argued that for most genebanks, genomics-based innovations will be highly driven by the demands of the research community and the plant breeding industry. Therefore, changes may be expected in the genebank's services to the user community, rather than to its housekeeping operations. These new services may include the distribution of specific research materials (such as mapping populations), providing access to allele-level information about genebank material, directed acquisition of novel variation and collaborative high-throughput phenotyping. These new services and the anticipated impact on genebank organisation and funding will be discussed and illustrated with examples from CGN's practice.

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## Adaptation of oilseed rape, barley and *Brassica rapa* to the future climate and increased [CO2]

Rikke Bagger Jørgensen<sup>1</sup>, Georg Frenck<sup>2</sup>, Teis Mikkelsen<sup>1</sup>, Cathrine Ingvordsen<sup>1</sup>

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With the present increase in the emission of green house gasses, the  $[CO_2]$  will double to app. 700 ppm and the average global temperature will increase 5-6 °C by year 2075. For this changed environment new crop cultivars are demanded. We analyzed the ability to adapt to this future environment in two crops, oilseed rape and barley. In an advanced phytotron (RERAF) environmental selection was applied over four generations to four cultivars/landraces of each species. Adaptation was also studied in a fast cycling line of *Brassica rapa*. The abiotic factors, temperature and  $[CO_2]$ , were elevated in single factor scenarios, and in a more realistic doublefactor treatment. A background level of 20-60 ppb ozone was applied in all scenarios that also received the same amount of water. The control treatment corresponded to present day Danish summer conditions.

In the generational selection applied for four generations, different trends were displayed by the species: Selection in **oilseed rape** at elevated  $[CO_2]$  led to higher reproductive output and this was independent of the applied temperature. Selection at elevated temperature (as single factor) did not result in changes of the production parameters. In **barley**, the reproductive output was decreased or unchanged in the final generation compared to the initial unselected material in all the climate treatments applied. In *B. rapa* seed yield was unaffected or decreased during selection, while vegetative biomass increased over the generations. These different responses to climate selection seen in oilseed rape, barley and *B. rapa* can possibly be ascribed to their different life history and different levels of genetic diversity - apparently some species possess the ability to adapt to fast environmental changes, while others might need a broadening of the gene pool to sustain the new environmental stress. Differences among the cultivars investigated in oilseed rape and barley indicated that a broader range of cultivars/landraces should be screened to pinpoint the most promising resources for breeding towards tolerance to climate change. We therefore also report here on climate screens of 32 oilseed rape cultivars. Our results on screening of 138 barley genotypes are presented elsewhere at this EUCARPIA meeting.

Acknowledgement:

The research was funded by NordForsk, FTP and DTU.

#### **References:**

Frenck G, LG Linden, TN Mikkelsen, H Brix, RB Jørgensen. 2011. Increased  $[CO_2]$  does not compensate for negative effects on yield caused by higher temperature and  $[O_3]$  in *Brassica napus* L. European Journal of Agronomy 35: 127-134. DOI 10.1016/j.eja.2011.05.004

Clausen SK, G Frenck, LG Linden, TN Mikkelsen, C Lunde, and RB Jørgensen, 2011. Effects of single and multifactor treatments with elevated temperature, CO<sub>2</sub> and ozone on oilseed rape and barley. Journal of Agronomy and Crop Science, 197, 442-453. DOI: 10.1111/j.1439-037X.2011.00478.x

Frenck G, LG Linden, TN Mikkelsen, H Brix and RB Jørgensen, 2013. Response to multi-generational selection under elevated  $[CO_2]$  in two temperature regimes suggests enhanced carbon assimilation and increased reproductive output in *Brassica napus* L. Ecology and Evolution in press.

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2 Food security







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## Genebanks and food security

N.R. Sackville Hamilton

Improving food security is a huge challenge. With increased demand for food as the human population increases, reduced supply of resources for food production, and increased constraints to productivity brought about by climate change, the challenge seems almost insurmountable. But we have the answer in the treasure troves that we call genebanks – a fact well known to us in the genetic resources community. We know the massive rapid adaptive radiation that has occurred in crop species over the millennia of traditional agriculture. We know that our genebanks contain the genes that breeders need to respond to almost any conceivable development objective, be it increased yield potential, improved nutritional quality, or adaptation to new climates, evolving pests and diseases, and new developments in sustainable agriculture. We are evangelists spreading the word that genebanks are the foundation of sustainable development in food production.

In this keynote address I will consider two questions. How successful have we been? And what do we need to do better to address future challenges?

Many countries in the centres of crop diversity of crops have been closing their borders to the export of their crop genetic resources. Perhaps they have heard our message, and decided they need to protect their own treasure. Yet while countries take protective measures, the financial constraints facing most genebanks suggest that we are failing to convince donors that genebanks are worth the cost. Moreover many breeders remain unwilling to jeopardise their hard-won genetic gains in their elite lines by crossing them with unproductive traditional varieties from the genebanks.

With the emerging technologies now available, the time has come to change from evangelistic rhetoric to action. We can now sequence whole genomes. We are working out what the genes do. We can find the functional allelic variants more easily than ever. We can begin to identify patterns in the distribution of genetic diversity. With this new knowledge we will be able to identify which specific accessions are most likely to meet any breeder's needs – the biggest challenge that has always genebank managers. And we will be able to design breeding strategies and markers that optimise the pathway from existing genetic resources to new genome-based ideotypes.

With seamless linkages from genebank through genomic research and pre-breeding to breeding, we now have the opportunity to resolve the political, financial and technical problems that have hindered effective use of genebanks in the past, and provide new sustainable solutions for food security.

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## Seeds of Discovery (SeeD): An initiative to systematically explore and mobilize novel genetic variation into maize and wheat breeding programs

Peter Wenzl, Sukhwinder Singh, Sarah Hearne, Marc Ellis, Martha Willcox, Carolina Saint-Pierre, Matthew Reynolds, Samuel Trachsel, John Hickey, Jiankang Wang, Juan Burgueño, Ky Mathews

To date only a minute portion of the vast diversity in genebank collections has been put to practical use in modern crop breeding. Recent advancements in DNA-sequencing technologies have created an opportunity to tap into, and unlock the value of these collections for the benefit of farmers, by assisting breeders to develop better cultivars more quickly. The Seeds of Discovery (SeeD) project, part of the Mexico-funded MasAgro initiative, strives to "see into seeds" to unlock the dormant genetic potential of maize and wheat genetic resources. SeeD is going to provide breeders with a toolkit of data/knowledge, germplasm, software tools and a genetic-analysis service, which together will enable a more targeted use of genetic resources in the development of high-vielding, climate-ready and resource-efficient maize and wheat cultivars (see http://seedsofdiscovery.org for more details). We are in the process of genome-profiling approximately 120,000 accessions from CIMMYT's wheat genebank and up to 40,000 maize landrace accessions (populations) from CIMMYT's and other Mexican genebanks, using genotyping-by-sequencing (GBS) methods. GBS minimizes ascertainment bias, an important feature when characterizing underexplored genepools. Accession subsets of varying size are being evaluated under field conditions for a set of priority traits including heat and drought tolerance, P and N efficiency, resistance to certain diseases and selected grain-quality characters. To lower the barriers to mobilizing novel genetic variation into breeding programs, we are developing 'bridging germplasm' with more breeder-friendly genetic backgrounds by systematically introgressing genome segments from genetically diverse accessions, partly enriched for favorable traits. In the case of wheat we are developing a 'Linked Topcross Panel' of 5,000 fixed lines, for which diverse genebank accessions are topcrossed to varying and partly overlapping combinations of elite parents, to enable joint linkage/association-mapping approaches, while also facilitating trait mobilization into breeding programs. In the case of maize, we have been characterizing a training population of 4,000 testcrosses across a range of environments and are about to initiate genomic selection-based introgression populations from the most promising accessions. The wheat and maize introgression populations, coupled with other data generated in SeeD, will facilitate a rapid, informed and targeted access to promising alleles by breeders.

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# Exploiting the INRA breadwheat collection to sample accessions used in French BREEDWHEAT project

#### DIDIER Audrey, DELOCHE Marion, BARDY Lionel and François BALFOURIER\*

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BREEDWHEAT is a "genomics to breeding" precompetitive national project built along a chain starting with the establishment of tools, methods and plant material for association genetics and ecophysiolgical studies of yield and quality components under biotic and abiotic stress, down to the evaluation of new breeding methods and the creation of prebreeding material that will support the selection of improved wheat varieties.

In the framework of this project, a specific task in work-package 3 (WP3) is dedicated to the definition of a worldwide wheat sample of 4600 accessions from the global INRA collection, in order to analyse genetic diversity in wheat germplasm and deliver two panels of 'easy to use accessions' for association analyses on biotic and abiotic stress tolerance in other work-packages.

The initial INRA bread wheat collection consisted on 11960 accessions originating from 108 different countries. Available data on this collection mainly consists on passport data: geographical origin (country, region, state, department, ...), status (landraces, breeding lines, cultivars, elite lines, ...), registration period, habit (spring, intermediate or winter type) and pedigree. Some molecular data, previously obtained on a part of the whole collection, highlighted the importance of both geographical and temporal effects in structuring the genetic diversity of this collection (Balfourier et al. 2007; Roussel et al. 2004, 2005).

The methodology used consists on sampling an optimal worldwide diversity sample mainly based on available passport data. The selected sample of 4600 accessions produces a balanced sample size between countries, regions and registrations periods, able to reflect the genetic structure of wheat genepools. This sample should permit to answer to the objectives of the BREEDWHEAT project. Furthermore, deliverable molecular data of WP1 (400.000 SNPs) on this sample of 4600 accessions should allow diverse population genetic analyses, such as multiple comparison of genetic diversity evolution (between countries, between regions within country, between registrations periods, between breeders, ...) and analysis of diversity at different time and eco-geographical scales.

#### **References:**

Balfourier F., Roussel V., Strelchenko P., Exbrayat-Vinson F., Sourdille P., Boutet G., Koenig J., Ravel C., Mitrofanova O., Beckert M., Charmet G. (2007) A worlwide bread wheat core collection arrayed in a 384-well plate. Theor Appl Genet. 114:1265-1275

Roussel V, Leisova L, Exbrayat F, Stehno Z, Balfourier F (2005) SSR allellic diversity changes in 480 European bread wheat varieties releades from 18040 to 2000. Theor. Appl. Genet., 111:162-170

Roussel V., Koenig J., Beckert M., Balfourier F.(2004). Molecular diversity in French bread wheat accessions related to temporal trends and breeding programmes. Theor Appl. Genet, 108: 920-930.

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## **Enhancing Global Capacity for Pre-breeding**

Chikelu Mba<sup>1\*</sup>, Elcio P. Guimaraes<sup>1,2</sup>, Clair Hershey<sup>1,2</sup>, Michaela Paganini<sup>1</sup>, Luigi Guarino<sup>3</sup>, Ehsan Dulloo<sup>1,4</sup>, Mario Marino<sup>5</sup>, Shakeel Bhatti<sup>5</sup> and Kakoli Ghosh<sup>1</sup>

1 Plant Genetic Resources and Seeds Team, Plant Production and Protection Division, Food and Agriculture Organization of the United Nations, Rome, Italy

2 International Centre for Tropical Agriculture, Cali, Colombia

3 Global Crop Diversity Trust, Bonn, Germany

4 Bioversity International, Rome, Italy

5 International Treaty on Plant Genetic Resources for Food and Agriculture, Food and Agriculture Organization of the United Nations, Rome, Italy

A diverse portfolio of well-adapted crop varieties is a veritable adaptive mechanism for cropping systems to cope effectively with climate change and variations. Ironically, the genetic base of the parental materials that are being used in developing the requisite novel crop varieties is extremely narrow for all the main crop plant species that underpin global food security. This pervasive situation, one of the causative factors for which is inadequately skilled human resources, may be the single most critical factor imperiling efforts to enhance the resilience of cropping systems worldwide. Pre-breeding, the activities for identifying desirable characteristics and/or genes from non-adapted materials -- that cannot be used directly in breeding populations -- and transferring these traits to an intermediate set of materials that breeders can use further in producing new crop varieties, can reverse this trend. The Global Partnership Initiative for Plant Breeding Capacity Building, convened by the Food and Agriculture Organization of the United Nations, has collaborated with various partners, especially the Global Crop Diversity Trust, Bioversity International and the International Treaty on Plant Genetic Resources for Food and Agriculture, in adopting a multi-faceted approach for building the capacity of scientists and policy makers in pre-breeding. For instance, a total of 134 scientists from 30 countries were trained in pre-breeding in four training courses held in three countries and one CGIAR centre. Additionally, an e-learning course in pre-breeding has been developed and along with its stand alone CD is publicly available (http://km.fao.org/gipb/e-learning/gipb-pre-breeding-course/en/). Multi-year pre-breeding projects for four crops have also been supported in four countries and one CGIAR centre. In similar vein, collaborative activities between germplasm curators and plant breeders for five crops have been facilitated in five countries. In recognition of the increasingly pivotal roles of the private sector in crop improvement, a consultation on public-private partnerships in pre-breeding has been held recently. We discuss the outputs from these interventions and share perspectives on future activities aimed at supporting capacity enhancements for increased introductions of novel genic variations into crop improvement programs.

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3 Nutrition and Health: Genetic diversity for health and nutrition



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## Genetic diversity for health and nutrition

Hilde Nybom

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My father worked as a scientist and breeder of fruits and berries at Balsgård, Sweden. As a young teenager in the emerging save-the-world movement of the 1960s, I reluctantly admitted that his job was probably somewhat better than being, e.g., the director of a lipstick factory! But certainly not good enough. Ideally, he (and later on his daughter) would instead have spent his time in developing countries, helping to feed the poor and heal their diseases.

As a brand new university student, I however attended an eye-opening lecture in 1970 given by Norman Borlaug in connection with his receiving the Nobel Peace Prize. He started by asking the audience who among us were lucky enough to be plant breeders or at least had the ambition to ascend to this state in the future. And then he went on to tell us how plant breeders were extremely important in relieving world famine! Hooray – this meant that there could be a world-saving aspect of my favourite subject, which just happened to be genetics. Still, of course I didn't see the work at home at Balsgård as being of much importance; surely fruits and berries were never going to feed any starving masses of the earth. This noble role belonged to the 'real' food crops like wheat, rice and maize.

Fortunately, our understanding of how different crops can contribute to human health and nutrition has improved vastly in recent years. It is not all about starch! Instead, we have experienced an increasing interest in plant-based products for human wellbeing at many levels, including the functional food concept. This has prompted research not only in the traditional crops but also in, for example, fruit, berries and vegetables where antioxidants and other health-related compounds are plentiful. Breeding cultivars with an improved ability to contribute to human health and nutrition is, however, a challenging proposition. Relevant characterization of available germplasm requires both biochemical and medicinal knowledge, while identification and mapping of responsible genes requires high-throughput molecular technologies. Here, I will provide an overview of recent developments from the perspective of a fruit- and berry breeder.

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## A large scale association study for aroma wheat components in bread wheat

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Wheat is consumed mainly as bread and the flavor or the aroma of bread is an important quality that can favor type of bread (wheat genotype) over another. A large collection of European hexaploid wheat (*Triticum aestivum* L.) lines was utilized to study the association between 2532 DArT polymorphic markers and the amount of 76 aroma components as traits. The wheat collection consists of 38 landraces and 147 cultivars, which were released during the period from 1889 to 2009 in different European countries. The 76 aroma components traits were analysed using Dynamic Headspace Extraction and Gas Chromatography- Mass Spectrometry.

A total of 267 putative QTL were found to be associated with 76 traits related to aroma. The putative QTLs ranged from 3 to 15.5 in the  $-\log_{10}$  (P) values. The number of QTLs per trait ranged from 2 to 43 with an average of 11 QTLs per trait distributed over the 21 wheat chromosomes. The number of traits per locus ranged from 1 trait to 23 traits per locus with an average of 3.3 traits per locus. Two markers were found to be associated with several traits related to aroma components, the first marker is wPt-009958, which is located at the position 18.8 on the chromosome 2A and found to be associated with 23 traits. The second is the marker wPt-008460 at the position 77 on chromosome 2B and found to associate with 19 traits. The strongest association was found between the marker wPt-004398 and p,Allyl anisole with  $-\log_{10}$  (P) value of 15.5

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## Genotypic variations in cadmium concentration of winter wheat grain – an opportunity for healthier bread

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Concentration of essential and detrimental trace elements in staple food crops is of great value for healthy human diet. The accumulation and distribution of trace elements in plants is affected by factors under genetic control (Grant et al., 2007.). Cadmium (Cd) is a detrimental trace element, a heavy metal with no biological function and highly toxic to plants, animals and humans. Therefore it is desirable to reduce concentration of Cd in crops used for human consumption. One way for achieving reduction is through plant breeding.

Winter bread wheat, as staple food, is also one of the Cd sources in human diet. Therefore, genotypic variation in uptake and Cd concentration in grain among cultivars is crucial for selection of low Cd concentration in winter wheat grain. A screening of genotypic variations in Cd concentration of winter wheat grain was conducted as a pot trial with 52 winter wheat cultivars. Cultivars originated from seven countries (Croatia, Hungary, Italy, Germany, Austria, France and Russia), registered in a period from 1936 to 2008. Cultivars from Croatia (34) originated from three different breeding centers. Trial consisted of two treatments regarding soil contamination: (1) non-contaminated soil and (2) soil contaminated with 20 mg Cd kg<sup>-1</sup> soil. Sample collection was conducted in flowering time (flag leaf) and in full maturity (seed). The results showed great differences in Cd concentrations of examined cultivars between and within countries of origin. Concentration of Cd in grain on non-contaminated soil ranged from 0.010 to 0.114 mg kg<sup>-1</sup>. On contaminated soil cultivars also showed significant difference, Cd concentration in grain ranged from 0.071 to 6.15 mg kg<sup>-1</sup>. High positive correlation existed between Cd concentration in grain and in flag leaf on contaminated soil. Results indicated that it is possible to lower the Cd content of winter wheat grain through cultivar selection and breeding. Several cultivars with different pedigree, origin and year of recognition can be marked as low Cd accumulators and use for selection of cultivars with low cadmium accumulation.

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## PGR of oat for gluten-free diets

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The gluten-free diet for celiac patients is based on functional foods which prevent pathological changes in people, genetically predisposed to this disease. Such a diet can be improved by including oats in it. However, there exist contradictory opinions on the existence of antibodies to oat proteins in the celiac patients' blood, and consequently toxicity of oat protein.

By means ELISA we study reactions between immunoglobulins (IGA and IGG) from 35 celiac patients and a group of healthy individuals, and proteins of different oat cultivars from the collection of the Vailov Institute of Plant Industry (VIR). Plant genetic resources are an inexhaustible source for improving functional foods.

Proteins of most oat cultivars react with antibodies of most patients almost as intensively as with wheat proteins. Only three cultivars (Argamak, Pushkinskii and Rhiannon) are distinguished by unusual reactions. Their proteins do not react with antibodies of half of celiac patients. It means that the immunogenic properties of these proteins differ from those of other cultivars. It is possible that proteins of the covered cultivar Argamak and two naked cultivars Pushkinskii and Rhiannon are non-toxic for some celiac patients. The discovered differentiation of both oat cultivars and celiac patients may help to individualize the diet and make more purposeful the search for cereal cultivars for producing functional foods for celiac diets.

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# Italian grape genetic diversity evaluation and exploitation

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The aim of this work has been to evaluate and characterize the levels of genetic variability in Italian grape germplasm and to understand the pattern of linkage disequilibrium (LD) and population structure for further genome wide association mapping studies. A collection of 440 grapes, belonging to Italian repositories and including wine varieties with wide range of cultivation areas, together with genotypes that are only locally cultivated or that are characterized by peculiar qualitative traits has been studied. After the establishment of an international Consortium, a 20K SNP Infinium array has been developed, starting from genomic sequences of 63 accessions of *V.vinifera*, 4 of *V.sylvestris*, 3 of *V.labrusca*, 3 of *V.cinerea*, 3 of *V.berlandieri*, 3 of *V.aestivalis*. A panel of SNP controls has been introduced in the array: more in details 5488 SNPs segregating in *V.vinifera*, 1300 segregating inside genus, 1200 species-specificic, 735 located in candidate genes has been selected.

This genotyping work can have significant implications for grape genetics and breeding and for fingerprinting and forensic phenotyping in Italian wine production chains, that are characterized by the presence of a very complex biological heritage in terms of grape genotypes.

Acknowledgements This work was supported by "Vigneto" project.

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4 Crop wild relatives. Multifunctional agriculture, climate change







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## Wild relatives in prebreeding: Fishing the genepool with dynamite

Hannes Dempewolf

Crop domestication has led to a severe reduction in genetic diversity within crops, as only a narrow range of diversity was selected by early farmers - an effect commonly described as the domestication bottleneck. But breeders need all the genetic diversity they can get their hands on to improve the world's crops. Many are therefore keen to reach back through the domestication bottleneck to make use of the diversity that can still be found in the ancestors and other wild relatives of crops (CWR). These taxa are increasingly recognized as being of key importance to breeding efforts in general, and those that aim to help adapt agriculture to climate change in particular. The list of possible traits that could be used to enhance crop adaptation to the world's new climates is extensive, including everything from enhanced root growth to faster grain filling. What reasons are there to think that such traits can be found in the generally unimpressive-looking wild and weedy plants that are the closest relatives of crops? We know that many CWRs grow in conditions of climate and soil that are marginal for the crop. We also know that many show marked differences from the crop, such as perenniality, fleshy roots and distinct phenology. Some of these are likely to be of important for adaptation. This talk will explore how wild plant biodiversity may turn out to be the crucial tool in our armoury to battle the perfect storm of ever-increasing world population, high input costs and a rapidly changing climate.

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## Extending use of the crop gene pool: developing national strategies for crop wild relative conservation and use

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The Convention on Biological Diversity (CBD) Strategic Plan (Target 13), the CBD Global Strategy for Plant Conservation (Target 9), International Treaty on Plant Genetic Resources for Food and Agriculture (Article 5) and the second Global Plan of Action for Plant Genetic Resources for Food and Agriculture (Priority Activity 4) each stress the imperative to actively conserve crop wild relative (CWR) diversity. This is associated with the dual Millennium Development Goals of wealth creation and food security, as CWR possess many beneficial traits that can be bred into crops to address changing environmental and market demands. Critically, the broad range of diversity found in CWR populations is increasingly important to buffer crops against the negative impacts of climate change but CWR genetic diversity itself is also being eroded and whole species lost as a result of climate change. Despite their recognized value for food security and wealth creation, CWR conservation has been widely neglected by national agencies; agricultural agencies having little responsibility for wild species conservation and ecological conservation agencies tending to focus on threatened habitats and endemic species. This has resulted in inadequate conservation of CWR populations both in situ and also in ex situ collections. However, countries which have ratified the CBD and ITPGRFA have a national and global responsibility to conserve their national CWR diversity and make it available for use in crop improvement programmes. But how can countries plan concerted conservation of their CWR genetic diversity to facilitate its exploitation? The development and implementation of a National CWR Conservation Strategy is an important step towards achieving this goal. This paper presents a methodology for developing such a National Strategy, the key steps of which are: (1) generation of a national CWR checklist, (2) prioritization of CWR diversity, (3) data collation and creation of the CWR inventory, (4) ecogeographic and gap analysis of priority CWR, (5) identification of threats to CWR diversity, (6) production of the National Strategy plan, (7) implementation of the Strategy, (8) integration of National Strategies into European conservation and (9) promotion of conserved CWR diversity for use in crop improvement. The methodology presented is illustrated with examples taken from the EC funded PGR Secure project that is currently establishing National CWR Conservation Strategies across Europe.

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# Ecogeographical approaches to characterize CWR adaptive traits useful for crop adaptation

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The sustained genetic erosion experienced by cultivated species has positioned crop wild relatives as the most important and readily available source of adaptive traits for crops. This is especially relevant in the context of climate change since crops will need to adapt to the abiotic and biotic changes in environmental conditions that are foreseen in the coming decades. As the number of populations of different crop wild relative species that can be taken into consideration for a particular crop can easily exceed various tens of thousands, a methodology to easily and efficiently screen the adaptive diversity inherent to these populations is needed. Taking into account that adaptive genetic diversity arises from natural selection acting upon differential limiting environmental scenarios, the ecogeographical characterization of wild populations provides the most efficient approach for generating representative collections of the available adaptive genetic diversity and selecting candidate populations for containing genetic adaptations for a particular trait. We are currently developing this approach by generating ecogeographical land characterization maps and ecogeographical core collections, testing the assumed correlation between ecogeographical diversity and adaptive genetic diversity, producing ecogeographically optimized seed collection designs and implementing focused identification germplasm strategies.

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5 Genetic resources and traits to address climate change







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## Genetic resources and traits to adress climate change

Key note speaker Andy Jarvis

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## The Focused Identification of Germplasm Strategy (FIGS): An approach to pre-breeding for water-limited environments

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The frequency and intensity of water deficit and heat stresses are expected to expand, particularly in already dry areas, due to climate change, so tools to identify germplasm with adaptation to dry and water limited environments will be increasingly needed. Prospects to assess and explore untapped plant genetic resources/biodiversity for traits to adapt to climate change such as drought effects are possible through new approaches such as the Focused Identification of Germplasm Strategy (FIGS). FIGS allows searching of large germplasm collections such as those of faba bean (Vicia faba L.), to find drought-related traits based on the paradigm that landrace crop varieties are likely to reflect the selection pressures imposed by the environment where it originates. To test this hypothesis a set of 201 faba bean accessions were selected from environments where there was a high likelihood of seasonal water stress and compared them to 201 accessions from environments that received higher levels of seasonal moisture. It was expected that traits related to plant moister status would differ between the two groups. Parameters related to stomatal function and water status were measured under well watered conditions. The dry set had significantly larger stomata, greater stomatal area per unit area of leaflet, higher relative water content and leaflet area, and also lower leaflet and canopy temperatures than the wet set. The difference between the two subsets was highly significant, indicating the potential of FIGS to search for the desired traits. The results also show that the discrimination between the two subsets can be based on pertinent physiological traits, such as leaflet and canopy temperature and relative water content, that can be used as surrogates for droughttolerance selection criteria in faba bean germplasm. Overall this study supports the assertion that FIGS could be an effective tool to search for traits related to adaptation to climate change.

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### Gene hunting at the Carlsberg Laboratory employing historic barley mutants

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Mutants are excellent tools for gaining insights into biochemical pathways, enzymatic reaction mechanisms, signaling networks and molecular processes in general. The Scandinavian barley (*Hordeum vulgare* L.) mutant collection located at the Nordic Genetic Resource Center, Sweden, is one of the largest barley mutant collections. With more than 11,000 entries, it is a fantastic resource for molecular studies connected to traits of applied and basic scientific interest.

In our research, we are especially interested in mutants with differences in day-length response, maturity, ripeness, chloroplast development, general plant size, culm length, culm stiffness and kernel size. The talk will focus on our recent results related to identification of genes causing a dwarf or semi-dwarf phenotype. Mutations in these genes are of importance for breeding of lodging resistant crop plants, an issue of growing importance in times of global warming.

Brassinosteroids effect cell elongation. Therefore, mutants deficient in genes encoding brassinosteroid receptors and brassinosteroid biosynthetic enzymes show a short-culm phenotype. Currently, only one gene (Uzu1) related to brassinosteroids have been characterized at the molecular level in barley (Chono et al. 2003 Plant Physiol. 133: 1-11). The uzu allele contains a single nucleotide polymorphism (SNP) in the coding region of the brassinosteroid receptor protein. The SNP causes an amino acid substitution resulting in a malfunctioning receptor protein, which causes a semi-dwarf phenotype. This uzu allele is widely spread in barley cultivars, but has negative agronomic properties. We screened a collection of dwarf and semi-dwarf barley mutants for their response to exogenous brassinolide in a so-called leaf-unrolling test. We identified novel alleles of Uzu as well as new barley genes deficient in brassinosteroid mutants.

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## Abiotic stress tolerance in 138 barley accessions tested in the future Nordic climate

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Climate change is progressing fast, why the development of high yielding cultivars suitable for the future growth environment is vital. We have screened 138 spring barley genotypes in the RERAF phytotron (http://www.increase-infrastructure.eu/Experimental sites/RERAF DK.aspx) under scenarios representing the climate in Northern Europe around year 2075. The abiotic factors; temperature,  $[CO_3]$  and  $[O_3]$  were constantly elevated in single factor treatments, and in a more realistic doublefactor treatment with temperature and [CO<sub>3</sub>]. Temperature was elevated by 5°C to 24°/17°C (day/night), [CO<sub>2</sub>] was doubled to 700 ppm and [O<sub>3</sub>] was 100-150 ppb; as control was present Danish summer conditions (19°/12°C (day/night), 385 ppm [CO<sub>2</sub>]. All climate scenarios were provided the same amount of water. The genotypes screened included landraces, old and modern Northern cultivars in addition to lines from Nordic breeding companies. The +5°C treatment reduced the yield by about 50% in average and the elevated [CO<sub>3</sub>] treatment increased yield by about 15%. No change was found when spring barley was grown under elevated  $[O_{1}]$ . In the doublefactor treatment, the vield stimulating effect of elevated [CO<sub>2</sub>] did not counteract the reductions in vield caused by the  $+5^{\circ}$ C, as the yield in the doublefactor treatment was reduced by about 30%. The reduced yield was caused by decrease in seed number and not amount of ears. The same trends in response to the abiotic stressors were found for biomass as for yield, but the biomass was generally less affected than the yield. Genotypes with static and dynamic yield stability were also identified. Response patterns of the individual genotypes to the abiotic stressors indicate large differences between the genotypes.

Based on the results an array of genotypes will further be tested under extreme heat, to select genetic resources favorable for future breeding to mitigate the effect of climate change on spring barley production.

Acknowledgement: The research was funded by NordForsk.

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## 6 Genetic endowments for reducing agriculture's footprints







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## Leveraging ecotypic diversity to improve breeding programmes in the forage grass *Lolium perenne*

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Natural genetic diversity is becoming increasingly important to understanding the ways in which we can improve plant breeding. Complex traits can be improved dramatically by bringing novel alleles from diverse ecotypes into breeding material. We surveyed a panel of ~700 natural and ~250 varietal accessions of *Lolium perenne*, an outbreeding perennial forage grass, across 1800 SNP markers using a custom Illumina Infinium assay. We show correlation of genotype with geographic origin, indicating that we can identify markers associated with adaptation to environment. In addition, we identify genetic signatures of selection for particular forage traits which may enable identification of the underlying genes.

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## Nitrogen use efficiency indices in old and modern barley genotypes

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Manufacturing nitrogen fertilizer is a high energy demanding process. Global phosphorus resources are perceptibly limited. Excessive use of these nutrients causes eutrophication of water systems. Hence, it is important that high yielding capacity is conjugated with efficient use (uptake and utilization) of nutrients. In the future, this is likely to be realized in growing environments and cropping systems that rely more on the sustainable use of production inputs and less on extensive use of inorganic fertilizers. Landraces and older varieties may provide genetic resources to improve yield, yield stability and nutrient use efficiency.

The aim of this study was to evaluate NordGen barley landraces together with a variety collection of old and modern cultivars for diversity in agronomic performance, yielding capacity and nitrogen use efficiency. All together 195 genotypes (72 NordGen landraces and 123 cultivars) were grown in two nitrogen regimes (35 and 70 kg N/ha) with three replicate plots. Trials were established (2011 and 2012) at MTT Agrifood Research Finland in Jokioinen. Just prior harvesting, 50 plants were randomly collected from the plots to determine vegetative biomass (mg/plant), grain yield (mg/plant), harvest index (HI, %), nitrogen harvest index (NHI, %) and nitrogen in grain (% dm, NIR) and in vegetative plant part (% dm, Kjehdahl) was measured. Following nitrogen use efficiency indices were estimated: nitrogen use efficiency (NUE; kg grain/kg available N), which can be divided into two separate components; a) nitrogen uptake efficiency (NUPE, kg plant N/kg available N) and b) nitrogen utilization efficiency (NUTE, kg grain/kg plant N).

According to the results of 2011 trial, breeding had clearly improved agronomic performance and yielding capacity. Average yield increase was + 16 kg grain/year. Yield improvement was associated with shorter stem length, improved lodging tolerance and higher HI. Breeding had consistently improved NUE and NUTE, whereas NUPE and NHI were virtually unchanged. NordGen landraces did not rank in high position in NUE, but in NUTE and NUPE some of them ranked in top 20 genotypes. Thus, landraces may provide some potential to be utilized in breeding programs aimed to improve nitrogen uptake or utilization.

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### Developing a European on farm conservation strategy for landraces

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The diversity between and within crop species has great, significant socio-economic value, as well as its heritage value. The bulk of plant genetic resource diversity in domesticated crops is located in their landraces (LR) because modern cultivars are largely bred for uniformity. Many legally binding regional and global conventions and treaties call for the safeguarding of on-farm diversity (generally referred to as conservation). Our aim here is to present some of the achievements of the ECPGR on-farm Working Group and the EC FPVII funded PGR Secure project (which was born inside the ECPGR group), which has raised regional and national awareness of LR and on-farm conservation concepts through meetings and publications. While members of the ECPGR WG agreed on a LR being 'a variable, named population which lacks formal crop improvement', the strict and continuous link with the territory and local people is seen as the critical trait that ensures LR maintenance, i.e. generally under marginal agronomic conditions, mostly the Southern and extreme Northern parts of Europe. In these regions LR on-farm conservation is maintained due to the value of the product that is obtained from LR, local culture and/or with educational activities. In other parts of Europe, in general, the geographic / cultural link is often absent and LR are often introduced from different areas or reintroduced from gene banks, often purposely to satisfy the demand of a more environmentally friendly agriculture. Currently the PGR Secure project is developing (a) a European strategy for LR on-farm conservation, (b) national LR conservation strategies, (c) the identification of the Most Appropriate Areas for on-farm conservation, (d) the assessment of genetic erosion and extinction risks and the development of a monitoring system, (e) the preparation of tools to aid the implementation of on-farm conservation of LR. These issues will be reviewed and discussed in the context of European Seed Legislation on Conservation Varieties.

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### Diversity in the perennial grasses species recommended for conventional and low input agriculture

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Permanent grassland are a source of healthy forage for a large group of ruminant animals. They also serve to conserve biodiversity, reduce environmental pollution, including nitrogen oxide and sulfur in the air and prevent soil erosion. Biodiversity include biotic variation at intraspecific genetic variation level, species diversity and ecosystem diversity. There are two different opinions regarding the impact of agricultural intensification on biodiversity. Some authors suggest that is that caused the extinction of many ecotypes and replacing them with new forms of crops. According to other authors the negative impact on the biodiversity is not observed, because the genetic resources *in situ* and *ex situ* form are included into the breeding programs and the wide variation within the newly created varieties could be maintained. Because of this Red Fescue, Meadow Fescue, Timothy-grass, Kentucky Bluegrass, Wood Bluegrass, Tall Meadow Oat and Redtop cultivars and ecotypes were evaluated in two traits using conventional and ecological fertilizers during 3 years. Resistance for biotic and abiotic stresses and plant morphology including leaf area and green and dray mass production were described. Based on the obtained results it was possible to conclude that from the marginal group of the grass species Redtop could be recommended for both type of agriculture.

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7 Intellectual property management and gene banks



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### Access to genetic resources and plant breeding the effects of IP and ABS obligations

Anke van den Hurk, April 2013

The first breeding activities were based on trial and error. The re-discovery of the laws of heredity from Mendel by Hugo de Groot in 1900 led to a next phase in the plant breeding sector where new varieties were developed through more advanced pedigree selection methods. After 1980 new biotechnology techniques were developed and started to find their way into plant breeding which resulted in more focused and more efficient breeding.

Once the breeding industry started to develop, breeders felt the need to protect their varieties to recover their development costs and be able to continue innovation. In the USA this resulted in an amendment to the patent law in 1930, which then allowed the granting of Plant Patents. This was only allowed for vegetatively propagated species excluding the edible roots and tubers. Europe did not follow the approach of the USA as they wanted to develop a system that would fit food security and allow breeders to use the protected material for new breeding activities. Several European countries supported the setup of UPOV in 1961 to protect the intellectual property rights of plant varieties, open for any country to become a member. Through the years, several new versions were adopted in respectively 1972, 1978 and 1991. This was done to remain in line with the technical progress in plant breeding.

With the entry of biotechnology techniques into plant breeding new forms of IP protection were desired. In the US this resulted into utility patents on plant varieties. Few countries have similar protection systems in which plant varieties can be patented. In the EU plant varieties cannot be patented. However, traits which may be expressed in more plant varieties may be protected by patents. With the patent systems genetic material was no longer always free for further research and breeding.

In the nineties developments of rights on biological diversity were addressed. In 1992 the Convention on Biological Diversity was adopted in which genetic resources cannot longer be seen as common heritage, but are under the sovereign rights of countries. For plant genetic resources for food and agriculture a specific treaty was developed, the International Treaty on Plant Genetic Resources for Food and Agriculture. In this treaty a multilateral system had been developed for a limited number of crops making the exchange of genetic resources possible under a standard contract, the Standard Material Transfer Agreement (SMTA). The development of access and benefit sharing (ABS) rules is often recognized as a reaction on the stricter IP rules on biological materials that were more frequently used from the eighties onwards.

Even though plant breeding has been improved and has become more sophisticated and targeted the need for access to genetic resources has remained the same. Without genetic variation it would not be possible to develop new varieties.

Until very recent any genetic material could be used for plant breeding without any obligations. Even when the UPOV laws came into force this need was recognized and a breeders' exemption was included in the law. The breeders' exemption allows breeders to make use of protected material

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without any agreement with the owner, as long as the end product that is put on the market is sufficiently different. Only very recently with the introduction of patents and ABS legislation, the access to genetic material is not free anymore.

This change has a large impact on plant breeders. A decision on using a genetic resource in a breeding programme is not any longer only based on biological aspects, but also on the legal consequences (including on the right to use and contracts) and with that the financial implications. To use a genetic resource, a plant breeder first needs to understand the obligations on the material. On the one side it needs to be known if there are restrictions with regard to IP protection and on the other hand it needs to be known what the ABS requirements are. With regard to IP, it may not always be clear to which genetic material IP protection is related. Currently databases are set up to get a better overview on which varieties are linked to which patents. It is good to realize that IP rights exhaust after a certain period.

With regard to ABS requirements, first of all the right authorities needs to be sought after which an agreement can be developed. Until present limited experience exists in this regard, especially with regard to access to genetic material in developing countries. Process for exchange are time consuming and mostly not have a positive outcome. In developed countries a lot of materials of genebanks are available under the SMTA, which has resulted in some exchange.

The fact that decisions to use genetic material are more complex may lead to lesser use of the genetic variation available, which may impact the maintenance of biodiversity per sé.

The change of rules on access to genetic material does not necessarily have only effect on the individual plant breeder, but also on the sector as a whole. To deal with the legal consequences may be too complicated for companies, especially small and medium sized ones that do not have legal staff. This therefore may result in corporate takeovers leading to less companies and more multinationals. Medium sized companies may therefore disappear and only small and large companies may remain. Also the difference between the breeding capacity between developed countries and developing countries may become larger, as starting up as a breeder or breeding community may be more difficult.

If the paradigm shift of free access to genetic resources for breeding to access on basis of agreements is an improvement for breeders, and the breeding sector and ultimately food security therefore remains a question.

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### The new Genebank CGIAR Research Program

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In 2012, the Genebank CGIAR Research Program ("CRP Genebank") was established to sustainably fund the conservation of plant genetic resources in CGIAR-held collections and to make them available to breeders and researchers in a manner that meets high international scientific standards, is cost efficient, is secure, reliable and sustainable over the long-term and is supportive of and consistent with the International Treaty on Plant Genetic Resources for Food and Agriculture. The CGIAR Centers hold and safeguard some of the largest, most important, most diverse, best documented and most used collections of the crops most critically important to global food security. They also have a unique history and international status that sets them apart from all others, and arguably gives their management an imperative and prominence unsurpassed by any other single undertaking in the CGIAR.

The CRP Genebank funds CGIAR centers to carry out a range of essential functions including acquisition, storage and maintenance, safety duplication, regeneration, multiplication, characterization, documentation, distribution and promotion of the use of genetic material. Increasing the overall efficiency and effectiveness must be achieved through a number of specific actions such as developing common databases, reducing unnecessary duplication, harmonizing quality assurance standards and performance reporting, and strengthening collaboration. A robust global conservation system recognizes participation by all relevant institutions, not just the Centers of the CGIAR.

The objectives of the CRP Genebank are:

- 1. Crop and tree diversity in international collections, holding international public goods under the International Treaty on Plant Genetic Resources for Food and Agriculture (Article 15), are secured in perpetuity, in long term and medium term storage facilities, and with safety duplication of accessions.
- 2. Conserved crop and tree germplasm is clean, available and disseminated. Accessions are introduced into collections, tested and cleaned of seed borne diseases, regenerated, multiplied and characterized.
- 3. The use of conserved crop and tree diversity is informed and facilitated, to manage the collections appropriately, and to ensure the potential use or value of the individual accessions is made evident. The development of GeneSys (www.genesys-pgr.org) is a new global accession-level information system that extends beyond the CGIAR-held collections.
- 4. Crop and tree diversity is conserved within a rationalized, cost-effective and globalized system. Conservation and use of germplasm is dependent on sustainable partnerships that strive to more effectively conserve the genetic diversity that will be required in the future.

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## The impact of international agreements on genebank management in Europe

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Most genebanks operate under government mandates. Therefore, international agreements relating to biodiversity and genetic resources that governments adopt impact on genebank management. Since 1993, the Convention on Biological Diversity (CBD) has been in force and has acquired near-complete global membership. In 2001, the International Treaty on Plant Genetic Resources for Food and Agriculture was adopted, since then ratified by most European countries. In 2010 the Nagoya Protocol on Access and Benefit-sharing followed, and implementation of this instrument in Europe can be expected in due time.

Growing realization that plant genetic resources represent an economic asset resulted in the introduction of Material Transfer Agreements (MTAs) by many genebanks. In consultations with their governments, genebanks had to develop distribution policies and access conditions. Whereas the CBD requires benefit-sharing arrangements for access to genetic resources under government sovereignty, no specific guidance was provided by the exact nature of such conditions, except that these were to be decided by the provider (or provider country) and recipient.

The coming into force of the International Treaty and the agreement of the Standard Material Transfer Agreement under the International Treaty resulted in a first internationally agreed Material Transfer Agreement containing elaborate access and benefit-sharing conditions in 2006, the Standard MTA. This SMTA has been adopted by many European collection holders.

The Nagoya Protocol has prompted the development of a European Union Regulation based on the concepts of due diligence, a Register of Best Practices for user communities and a List of Union Trusted Collections, in which genebanks can be incorporated provided to fulfil certain standards regarding access and benefit-sharing, in particular the legitimate acquisition of genetic resources for their collections.

All these policy measures impact on genebank management. Genebanks need agreed access and benefit-sharing policies and internal quality systems to implement these policies, documentation of agreed MTAs and reporting on such MTAs to national and international checkpoints. This paper analyses the current state-of-affairs regarding the extent of response by genebanks to such policy requirements and the mechanisms by which genebanks can cope with these requirements.

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## Plant Genetic Resources: Characterization and Utilization: a journal with a future

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Plant Genetic Resources: Characterization and Utilization is the successor to the journal Plants, Varieties and Seeds, published for many years by the National Institute for Agricultural Botany (NIAB) in Cambridge. It was launched in 2004, and has produced three issues per year on a regular basis since then. Now published by Cambridge University Press and with an international editorial board, the journal will this year be allocated its official impact factor by Thomson Reuters ISI. As suggested by its title, its mandate takes in research in the areas of germplasm collection, conservation, characterization and exploitation, so represents an ideal forum for *in situ* and *ex situ* conservators. A feature of *PGR:C&U* is its rapid manuscript turnaround time. We average about one month between manuscript submission and the initial verdict, and about three months between submission to final decision. Accepted papers are routinely mounted online within days of receiving the author's galley proof, and will generally appear in print within six months.

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### Public Private Partnership in Pre-Breeding: Combining Knowledge from Field and from Laboratory for Pre-breeding in Barley

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In all the Nordic countries the commercial breeding programs have a long term focus on the current main diseases of barley. Several research projects have been undertaken, some of them inter-Nordic, and in a few cases DNA markers have replaced disease tests in commercial breeding, but the potential is still underutilized. Therefore, there is a need for a deeper and more structured collaboration to prepare for the future. In addition, the consequences of the climate change, in combination with the goal to reduce fungicide use for sustainable agriculture and food safety, justifies further Nordic projects.

The main goal for this pre-project is to lay the foundation for effective cereal breeding for disease resistance and harvest stability in changing climatic conditions capable to meet current and future challenges in the Nordic region. This will be done by developing physiological, genetic and molecular knowledge, tools and germplasm for the future and better adaptation to growing conditions. More specifically, the primary objective is to develop and use available molecular markers to screen current breeding material for traits related to the most important diseases, as well as important agronomic traits, such as earliness, lodging and straw/ear breaking. For this purpose, 180 barley lines (30 from each participating breeding company) have been phenotyped for the specific traits in field trials in all Nordic countries and genotyped with 9K iSelect SNP markers resulting in 7864 DNA markers. An association analysis combining genotypic data with phenotypic data has been conducted for phenotypic data from first year field experiments. Preliminary results have identified markers for disease resistances and agronomic traits important for climatic adaptation. The pool of 180 barley lines will be available for all participants to use in their respective breeding programs at the end of the first phase of the project. In addition, a comprehensive database has been established which contain information on published DNA markers linked to agronomic traits. This will significantly increase the efficiency of the breeding process. Further, our aim is that this pre-project will lay the foundation for a long-term and effective collaboration between the participating Nordic breeding companies in barley pre-breeding.

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## The Nordic Public-Privat Partnership (PPP) for pre-breeding in perennial ryegrass (Lolium perenne L.)

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In Northern Europe the expected climate changes will result in new growth conditions for forage production due to an extended (1-3 months) growth season combined with milder and rainier autumns and winters. Perennial plants like forage grasses and legumes show local adaptation to temperature and photoperiod. Important physiological processes governing plant phenology (vernalization, cold acclimation, heading and flowering) are determined by temperature and photoperiod and their interactions. This in turn determines winter survival and seasonal yield distribution. The seasonal photoperiod does not change with the climate changes that cause the temperature to rise. This raises a need for new ideotypes with a different set of physiological traits than the present cultivars. An increased risk of frost injury in spring in some locations, and increased frost exposure during winter in others given that the current cultivars are used are some of the expected consequences. A pertinent question is thus whether the available germplasm and active breeding populations are sufficiently broad, or exotic materials should be identified, introgressed by crossing and recombined, to serve as new genetic resources for development of cultivars for the future climate at higher latitudes.

Perennial ryegrass (*Lolium perenne* L.) has superior feed quality and productivity under frequent cutting regimes, and is expected to expand further north due to milder winters with shorter periods of snow cover. The main weaknesses are susceptibility to low-temperature pathogens, inadequate growth cessation in the autumn to allow for sufficient cold hardening and winter survival, with low persistency as the result. In order to prepare for the predicted climate changes a PPP for pre-breeding programme aimed at selecting plant materials of perennial ryegrass for the development of cultivars with a suitable adaptation to future climates in the Nordic countries was initiated in 2012. Breeding of perennial grasses is a long-term activity which require many years from the starting exploitation of germplasm until the release of improved cultivars. The on-going and planned work of the perennial ryegrass pre-breeding project will be described.

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### **Prebreeding for Future Challenges in Nordic Apples**

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The Nordic Council of Ministers (NMR) has set up a Public Private Partnership (PPP) for prebreeding of plant crops in 2011. The purpose of the PPP is to support the development of Nordic plant breeding, satisfying the long-term needs of the agricultural and horticultural industries. In a PPP pre-breeding project on apple (2012-2013) we search for sources of resistance to *Neonectria ditissima* (causing European canker on trees) and *Penicillium expansum* (causing blue mould on fruits). Cut shoots of 50 cultivars are wounded (bud wounds) and inoculated with conidia of *Neonectria ditissima*, and lesion development monitored. Apple fruits of 80 cultivars are wounded and inoculated with conidia of *Penicillum expansum*, and diameter of lesions measured after 6 or 12 weeks of storage. The obtained results will be used in further work towards genetic dissection of complex resistance to these diseases. DNA samples will be prepared for all of the phenotyped cultivars, and analyses of genetic background will be undertaken and QTL markers developed in cooperation with the EU-funded Fruitbreedomics project, to which Swedish University of Agricultural Sciences is an 'invited partner'. Within the PPP project a network of Nordic breeders of fruit and berries is developed, in order to define and develop projects that meet with the challenges for Nordic fruit and berry production.

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### Safety duplicating global *ex-situ* collections: Taxonomic and institutional representation in the Svalbard Global Seed Vault collection.

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Five years after the opening the Svalbard Global Seed Vault conserves safety duplicates of 774,601 accessions of plant genetic resources for food and agriculture (PGRFA). The 53 genebanks that have made use of the Seed Vault connects institutions across political differences and economic status of their location countries. This global collaboration is in line with the goal of the International Treaty for Food and Agriculture (ITPGRFA) which states that each contracting party shall "cooperate to promote the development of an efficient and sustainable system of ex situ conservation ... " (FAO 2001) and the Global Plan of Action for PGRFA which calls on all FAO member countries to develop "a rational, efficient and goal oriented, economically efficient and sustainable system of exsitu conservation and use ..." (FAO 2011). The global back-up collection in the Seed Vault currently covers more than 800 genera of crops and crop wild relatives. The twelve largest genera in terms of number of accessions stored are those of wheat (Triticum), rice (Oryza), barley (Hordeum), sorghum (Sorghum), common bean (Phaseolus), maize (Zea), cowpea (Cicer) cowpea (Vigna), triticale (Triticosecale), pearl millet (Pennisetum), soybean (Glycine) potato, tomato and eggplant (Solanum), all with more than 15,000 safety duplicates in the Seed Vault. The majority of the Seed Vault collection is deposited by National Agricultural Research Organizations, and currently there are more participating genebanks from developing countries than from developed countries. The largest safety duplication collections in the Seed Vault are deposited by the centers of the Consultative Group on International Agricultural Research (CGIAR) and the crop genera for which the CG centers hold in-trust for the world community under Article 15 of the ITPGRFA are those that are best represented in the Seed Vault. In this paper we present maps of origin of the global collection at Svalbard for selected genera. The number of accessions from different countries is associated with the centers of diversity for the different crops in the genera as well as institutional location. Finally, we assess coverage and gaps of genepools of selected crops in the current Seed Vault collection.

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## Abstracts

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1 Genomic of gene banks

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### Application of DNA fingerprinting methods for fruit crop genetic resources characterization in Latvia

poster p1-01

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A diversity of fruit crop varieties is available in Latvia, which consists of landraces and selections of local breeding as well as germplasm that result from years of plant material exchange among different countries and breeding institutes. Main feature of fruit crop germplasm in Latvia is adaptability to the local climate and growing conditions. Base collection of fruit crop genetic resources is maintained at the Latvia State Institute of Fruit-Growing. Presently this collection comprises about 2700 accessions of 19 fruit crops; 724 accessions are designated as national genetic resources. Conservation of germplasm itself has a little value without characterization and further utilization of the stored plant material. To intensify these activities DNA based fingerprinting has been implemented in the characterization of germplasm. In general, two main groups of molecular markers have been utilized for PGR characterization: markers for identification of accessions, detection of the structure of genetic resources collection and relatedness of available plant material, and gene specific markers, subsequently applicable for Marker Assisted Selection (MAS). At the moment genotyping methods based on SSR and RAPD markers for eleven fruit crops have been implemented for use in the plant material identification, True-to-Type verification as well as evaluation of genetic diversity and internal collection structure. These marker sets have been harmonized with ECPGR WG recommended ones to ensure data compatibility with international data bases. Presently 723 accessions of nine crops have been genotyped. Gene specific molecular markers have been applied for apple (resistance to scab), sweet cherries and plums (Sf – selfincompatibility gene), which ensure essential information for germplasm utilization in MAS and breeding.

Key words: germplasm, molecular markers, MAS, SSR, RAPD, gene specific

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## Using genebank accessions for discovery loci determining pre-harvest sprouting and dormancy in wheat – an association mapping approach

poster p1-02

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Pre-harvest sprouting (PHS) is a phenomenon of cereal crops when germination of grains occurs in the spikes before harvest and therefore it is a big problem in wheat production. It reduces crop yield to the point of a total damage of the harvest. Due to climate change PHS can appear more often in future. Seed dormancy can prevent sprouting. Searching for dormancy and sprouting genes can help to avoid these effects. As complex traits with high genetic variation it can be assumed that both traits are controlled by multigenes.

In order to detect marker trait associations (MTAs) a population based on 183 genebank accessions of *Triticum aestivum* primarily selected for seed longevity was used. 2,134 mapped DArT (Diversity Array Technology) markers are available for an association mapping approach. For phenotype-genotype association analysis the software programme TASSEL 2.1 was used to calculate associations between the markers and each trait. Two model approaches, the general linear model (GLM) and the mixed linear model (MLM), were employed in order to determine MTAs. A MTA was considered only if it occurs in both models on a p<0.01 level.

Many MTAs could be detected on all seven chromosome groups of wheat. Especially, the known regions on chromosome 3A and 4A were confirmed. Via a candidate homologues search putative functions could be found. A homoeologous region for PHS and dormancy on the long arm of chromosomes 1A, 1B and 1D near to the centromere was detected. For the region of chromosome 1B a thionin precursor is known which is involved in plant reactions against microbial infections and toxic to plant microbial pathogens. Thionins located in the endosperm of wheat are encoded by genes on all three group 1 chromosomes. On chromosome 3A the *viviparous1* gene is located which is associated to pre-harvest sprouting and dormancy. On chromosome 4A a protein is detected which belongs to the aquaporin family. Aquaporins are responsible for water flow through the cell membrane. It can be concluded that dormancy and pre-harvest sprouting are very complex traits regulated by multigenes.

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### Comparative genetic diversity analysis of common beans grown in Kyrgyzstan using molecular and morphological markers

poster p1-03

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Common bean (Phaseolus vulgaris) is an important export crop in Kyrgyzstan since the end of the 20th century. Kyrgyzstan produces around 70,000 metric tons of common beans per year, which is a moderate amount when compared to its production in other major common bean producing countries. This Central Asian country is among the top ranking bean exporting countries in the world with estimated annual income of 19,949,000 US dollars. Genetic diversity analysis of common bean populations is useful for breeding programs, as it helps to select appropriate genetic material to be used. Twenty-seven common bean accessions originated from both the Mesoamerican and Andean gene pools were analyzed using simple sequence repeat (SSR) molecular markers and qualitative morphological traits. Nine polymorphic microsatellites and 13 morphological traits were used in this analysis. The similarity matrices generated from the molecular and morphological data were well correlated (r = 0.49, significant at p < 0.01). The cluster analysis of both data sets grouped the 27 accessions according to their gene pools of origin: 15 belong to the Andean and 12 to the Mesoamerican gene pool. The analysis revealed that the Andean accessions were less diverse than the Mesoamerican accessions. Higher genetic diversity was revealed by SSR markers when compared to that of qualitative morphological traits, as shown by Shannon diversity index (I<sub>ssp</sub> = 0.08; I<sub>morpho</sub> = 0.05). Overall, this study revealed that both SSR and qualitative morphological markers are suitable to assign modern cultivars to their gene pools of origin.

Keywords: Qualitative morphological traits, Phaseolus vulgaris, SSR

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## Practical use of molecular markers of pungency in breeding program of pepper (*Capsicum annuum* L.)

poster p1-04

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Marker-assisted selection (MAS) is a useful additional tool in modern plant breeding programs to optimize selection efficiency especially when monogenic traits are involved. For breeding purposes, molecular markers enable analysis of a large number of samples, detection of desired alleles and haplotypes at early stage of plant development, and reduction substantially the breeding period when biennial crops are involved.

Pungency of pepper (*Capsicum* spp.) is due to accumulation of capsaicinoids, of which capsaicin is the most pungent compound. The capsaicin biosynthesis is controlled by the *Pun1* locus, which encodes a putative acyltransferase. The recessive allele of this locus (*pun1*) contains a deletion that results in non-pungency. The co-dominant PCR-based markers distinguishing these alleles were developed (Stewart et al., 2005; 2007).

The  $F_1$  hybrids and  $F_2$  segregating population from the cross between non-pungent and pungent pepper cultivars were screened by molecular markers for pungency. Hybridity of the  $F_1$  plants was confirmed. At the stage of seedling the 106 plants of  $F_2$  population were analyzed by allele-specific markers for homo- and heterozygosity of *Pun1* locus. The non-pungent homozygous (*pun1/pun1*) genotypes as well as the pungent homo- and heterozygous (*Pun1/Pun1* and *Pun1/pun1*, respectively) genotypes were determined. Dou to dominancy of pungency, the phenotype-based distinguishing of the heterozygous genotypes (*Pun1/pun1*) from homozygous genotypes (*pun1/pun1*) is not possible. For the next stage of crossing only pungent homozygous (*Pun1/Pun1*) plants were selected, that reduced in three times the volume of breeding material involved in the breeding program.

Molecular-based genotyping at the seedling stage can be used efficiently in breeding to complement or to replace part of phenotypic selection.

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### Association mapping in complex synthetic and common hexaploid wheat collection using DArT markers

poster p1-05

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A collection of synthetic and common hexaploid wheat (Triticum aestivum L.) were used to study the association between major wheat diseases in Denmark and 1192 mapped polymorphic DArT markers. The 184 wheat lines collection composed of 122 synthetic wheat lines and 62 common wheat lines. Wheat diseases scored in three years and at three locations in field and semi-field experiments. Diseases included in this study are powdery mildew (Blumeria graminis); yellow rust (Puccinia striiformis); brown rust (Puccinia triticina), Fusarium head blight (Fusarium spp.) and mycotoxins (DON (Deoxynivalenol), NIV (Nivalenol) and ZEA (Zearalenone) produced by fusarium. A total of 14 independent disease/year traits were included in the association analysis and yielded more than 150 DArT markers with  $-\log_{10} P$  score equal or higher than 3. However, the complex population structure of the collection made it difficult to separate the true from false positive association with the DArT markers. Therefore we examined selection criteria to screen the obtained QTL based on 4 magnitudes: 1) The LOD score value of the associated marker on the trait 2) The standard deviation of the effect, 3) having more than one DArT marker with relatively high LOD score flanking the DArT marker associated with the trait, 4) a clear difference in disease reaction between accessions that have "1" allele and accessions that have "0" allele over most plant groups (synthetic and common) aiming at avoiding the effect of genetic structure of the plant collection over the association. By applying these criteria we reduced the number of putative QTLs to 21 OTLs. Out of the 21 putative OTLs, 12 were found novel OTLs and 9 OTLs were positioned on the same chromosomal location of known QTLs/Genes, which validate the criteria we used to screen out the false positive QTLs.

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### Genetic diversity in Kyrgyz apple

poster p1-06

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Species that grow in the forests of Central Asia show great diversity. Russian botanists found wild trees bearing the full range of forms that we know as the domestic apple. Particularly rich are apple forests around Alma-Ata in Kazakhstan, which means 'Father of Apples'. The domestic apple, together with many other temperate fruit crops originated also in the forests of Kyrgyzstan. The Russian plant geneticist Nicolai Vavilov and his colleagues, during their first visit to the forests of Kyrgyzstan, found wild apple species. Even today large areas of wild fruit trees can be found in the forests, especially in southern Kyrgyzstan. Local germplasms are the most important carriers of valuable features and the raw material for apple improvement and breeding new cultivars. Selective adaptation of fruit species to the conditions of Kyrgyzstan began in 1947. The Botanical Garden of the National Academy of Sciences KR had 7000 hybrids. Around 1975 there were 3500 apple hybrids. The main objective of our study was to assess and identify genetic diversity of apple (cultivars and wild relatives) in Kyrgyzstan. A collection trips were conducted from June to August 2012 to the southern part of Kyrgyzstan, especially around Uzgen and Jalal-Abad regions and the northern parts, Chui region and Issyk-Kul region. The Issyk-Kul region has unique conditions for growing high quality of apples and thus most of the samples were collected in this region. Leaves of 55 wild and cultivated apple forms were collected including two wild-growing species, M. Kirghizorum and M. Niedzwetzkyana. The collected materials are being characterized with microsatellites (SSR) at the Swedish University of Agricultural Sciences (Alnarp, Sweden). SSR markers used for characterization of European collections within the EU-project 'Fruit Breedomics', are applied in our study, thus allowing comparisons of Kyrgyz apples with apples from Europe. Molecular characterization together with the description of morphological traits will help to develop effective preservation strategy for apples in Kyrgyzstan as well as facilitate effective utilization of genetic resources in apple breeding.

Keywords: Central Asia, DNA-markers, wild apples

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### Cytomolecular genome analysis of Triticum monococcum

(Fishing in the gene pool using FISH)

poster p1-07

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*Triticum monococcum* represents an important source of useful genes and alleles that would be desirable to use in wheat breeding programmes. The well defined landmarks of A<sup>m</sup> chromosomes would accelerate the targeted introgression of *T. monococcum* chromatin into the wheat genome.

The fluorescence *in situ* hybridization (FISH) using the repetitive DNA probes pSc119.2, Afa family and pTa71 shows that the pSc 119.2 probe is not suitable for the identification of A<sup>m</sup> chromosomes. In contrast, the whole set of A<sup>m</sup> chromosomes (especially the chromosomes 1, 4, 5 and 7) could be discriminated by the hybridization pattern of pTa71 and Afa family. *In situ* hybridization with microsatellite motifs (GAA, CAG, AAC, and AGG) proved that SSRs represents additional landmarks for the identification of A<sup>m</sup> chromosomes. The most perspective SSR probes were the GAA and CAG motifs which clearly discriminate the 6A<sup>m</sup> chromosome and their use together the Afa family and pTa71 probes allow the perfect identification of the whole set of A<sup>m</sup> chromosomes.

In conclusion, the fluorescence *in situ* hybridization using repetitive DNA probes Afa family and pTa71 and SSRs probe will be able to identify the  $A^m$  chromosomes of *T. monococcum* and possibly to discriminate them from the  $A^u$  chromosomes in the polyploid wheat background.

The research works leading to these results have received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under the Grant Agreement n°245058-Solibam, and from National Science and Technology Office project (*TECH\_08-A3/2-2008-0423 - ALKOBEER –* Hungary).

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## Molecular cytogenetic characterization of perennial *Thinopyrum* species with the aim of description pre-breeding materials produced in Martonvásár

poster p1-08

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Wild relatives of cultivated wheat in the tribe *Triticeae* represent a rich potential source of genetic variation for many agriculturally significant characteristics. Perennial species such as wheat grasses includes diploid and polyploid species, containing genomes that are non homologous to those of wheat, are important as tertiary gene pools for wheat improvement. Species belonging to the *Thinopyrum* genus are known to possess genes conferring resistance to various diseases and are capable to produce hybrids with bread wheat.

Understanding the organization of the genomes in the *Thinopyrum* genus and their phylogenetic relationships with other related genomes will greatly facilitate the utilization of these species for transferring agronomically useful genes into bread wheat. The rapid, accurate identification of prebreeding materials can only be achieved by generating detailed FISH-based karyotypes of individual genomes by using different molecular cytogenetic probes. Complete FISH pattern description of the individual chromosomes of diploid *Thinopyrum* genomes is still missing and makes it difficult to analyze the progenies of intergeneric hybrids or identify the incorporated alien DNA segments in a wheat background.

Recently, the diploid EE genome of *Elytrigia elongata* detailed FISH chromosome pattern using repetitive DNA clones have been developed (Linc et al. 2012). FISH based karyotypes of diploid genotypes carrying JJ, PP, S'S' and NN genomes is under construction. Repetitive DNA sequences and trinucleotide microsatellite contigs have been used for mcFISH experiments.

Pre-breeding materials have been developed in Martonvásár by crossing the leaf rust resistant BE1 partial amphiploid (containing *Thinopyrum ponticum*, Sepsi et al. 2008) with several wheat genotypes. Stable, leaf and stem rust resistant lines with 42 chromosomes were selected after 12 generations. Diploid progenitor genomes of *Th. ponticum* (such as JJ and J<sup>s</sup> J<sup>s</sup>) have been used as labelled molecular cytogenetic probes for GISH (genomic *in situ* hybridization) experiments in order to detect alien chromatin in the wheat background.

This research was supported by the Hungarian National Grant (OTKA) nr. 104382 and a Janos Bolyai research fellowship to G.L.

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### SSR analysis of sugar beet (*Beta vulgaris* L.) parental forms of different origin for predicting possible heterosis effects

poster p1-09

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Pre-breeding is a first step in the use of genetic diversity from wild relatives and other unimproved germplasm. For cultivated sugar beet, many valuable for breeding improvement traits imported from wild beets are known. Monogermity, CMS, salt tolerance, resistance to beet necrotic yellow vein virus BNYVV, Erysiphe betae, Rhizoctonia solani, Cercospora beticola came from Beta (B.) vulgaris subsp. maritima, genetic male sterility from B. macrocarpa, frost tolerance, resistance to curly top from B corolliflora, seed shattering from B. trygina, resistance to Erwinia spp., Heterodera schahtii from Patellifolia procumbens (1). Beet accessions with such traits/genes are conserved in regional genebanks. Sugar beet is an obligate cross-pollinated species with its breeding based on crosses between diploid male sterile (CMS) lines and mainly diploid pollinator populations. The most labor and time-taking stage in a hybrid production program exploiting the phenomenon of heterosis, is the identification of parental forms. Information on the genetic diversity and distance between the breeding lines, and the correlation between genetic distance and hybrid performance are important for classifying the parental lines, heterotic groups and predicting hybrid performance. In pursuance of the heterotic pattern concept as a specific pair of heterotic groups (defined as populations/lines of related/unrelated genotypes from the same/different populations which display a similar combining ability when crossed with genotypes from other germplasm groups) that express in their crosses high heterosis and high hybrid performance (2), we took for SSR analysis 150 sugar beet plants representing 4 monogerm CMS lines with resistance to rhizomania and cercosporosis from Washington State University Regional Plant Introduction Station (pool # 1) and 220 sugar beet plants representing 5 populations of multigerm pollinators mainly from European breeding programs, including one gynogenetic DH line created by ourselves (pool # 2). Six SSR loci localized on 6 different chromosomes were screened in all plants of 9 beet populations. Alleles number and size in bp, their frequencies in populations were scored, heterozygosity rates in parental forms were estimated. Breeders selected plants with better performance of 1 and 2 year of vegetation valuable beet plant traits. SSR data describing pedigree were clustered showing populations structure, genetic distances between populations and clear difference between two pools. Predictions for parental pairs to cross were done. Hybridization will take place this year.

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## Molecular markers for botanic identification among Brassica species

poster p1-11

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Collecting wild relatives of *Brassica* (n=9) species is sometimes puzzling as the morphology within a species is variable and often descriptors do not completely discriminate between the different taxa. This has lead to dubious taxonomical determinations and some have been discussed for many years without any firm conclusions. We therefore decided to use simple DNA fragment analysis to bring more information into the discussion.

We have identified four complicated cases deserving investigation:

- "Wild" kale on Helgoland, Germany
- "Wild" kale in Rødvig, Denmark
- "Wild" kale at Monte Conero, Italy
- "Wild" kale on Isola di Ponza, Italy

Our approach was to compare the sampled populations with well known populations of agreed taxonomical status from classic locations. All accessions were collected or requested from genebanks. DNA was isolated, PCR amplified using inter simple sequence repeat (ISSR) markers. DNA fragments were separated on a gel which was stored as electronic files and converted to binary matrices using the Cross Checker software. Subsequently the data were analyzed using GenAlEx and AFLPop. Total genetic diversity and diversity within and between populations were determined. The structures of populations were analyzed using principal coordinate analysis and assignment analysis. Our results show with a few exceptions that ambiguity in determining taxonomical status of some populations also exists on the molecular level; however our experiments add new perspectives to the discussion of taxonomy of *Brassica* populations.

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### DNA marker screenings in fruit tree gene banks

poster p1-12

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Maintenance of live plant collections is extremely expensive but necessary for many crops like the vegatatively propagated fruit trees. Identification of accessions in fruit tree collections with simple sequence repeats, SSR, have shown that a very large number of the trees could be mislabeled. Typically, 20–40% mislabellings have thus been reported in e.g. apples and pears. More information about the 'true' name of the analysed trees can be obtained if the SSR data are compared with data for material in other gene banks. This, however, requires that the same set of markers is used in the different laboratories. The ECPGR, European cooperative program for plant genetic resources, have suggested standard sets of SSR loci for apples, pears and cherries. In a previous evaluation of 86 Swedish pear cultivars, we were able to verify the labeling of some cultivars but also to detect synonyms and mislabelings by comparing our material with a large pear collection at Brogdale, UK, evaluated with the same set of markers.

Recently, there has been an upsurging interest in phenotyping plant gene banks for traits such as resistance to fungal diseases and fruit quality. This is an important step in raising the status and practical utilization of plant genetic resources collections. However, access to properly identified material is a prerequisite for producing meaningful information from the phenotyping as well as from DNA marker data on traits of economical interest and/or of interest for plant breeders.

In 2011, we attempted to genotype 52 cherry samples (32 sweet cherry and 20 sour cherry) with SSR markers suggested by the ECPGR. Unfortunately, only six loci produced useful data. It seems that only 57% of the trees included in this study were correctly labeled. Parentage could be verified in some cases where both parent and offspring cultivars were analysed.

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# A diversity study of the Danish and Norwegian collections of hops (*Humulus lupulus* L.)

poster p1-13

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Hops (Humulus lupulus L.) have been cultivated in the Nordic region since the medieval times for conservation and flavouring of beer. Over the past years single plants have been collected from gardens and villages, as well as from breeders and companies (e.g. Carlsberg), and kept in clonal archives. Long-term conservation of clonal material is a costly issue, and to reduce the conservation costs true duplicates should be excluded. Therefor morphological and molecular characterisations of the clones have been carried out. The morphological characterization was done by Dragland et al. (2003), while the DNA study was done in 2010 by Nordic Genetic Resource Centre. The DNA study included108 samples from clones kept in germplasm collections in Norway and Denmark, with a few clones from Sweden and some newly collected clones from medieval sites in Denmark. The primers were selected based on information given by Patzak (2001) and studies carried out at Nordic Genetic Resource Center. AFLP analyses with the following primers were used: E38/M47, E36/M49, E37/M62, and E37/M47, resulting in 41 polymorphic bands. The results show that the collections hold clones that all are genetically unique. A correlation between the genetic pattern and morphological characters as well as to the geographical origin of the accessions was done. Regarding morphology the Danish and the Norwegian collection both have clones with the green and the red shoot coloration represented. The Danish collection had a few clones with green shoot ribs, while all the clones in the Norwegian collection had red shoot ribs. The clones in both collections showed variation in leaf blistering, leaf size, cone size, and cone form.

As far as this study shows, the decision on exclusion of clones from the collections is still open for debate. Our study has shown that all the clones are unique. Furthermore, we are aware of the conclusions from inventories on monastery ruins and medieval sites showing that hops can be found as plants from a possible relict cultivation (Løjtnant 2007). The cultural aspect should definitively be included in the debate, and a broad approach, including morphology and genetics, but also cultural history and cultural value of the plants should be highlighted.

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# Genetic diversity of date palm (Phoenix dactylifera L) in Sudan

poster p1-14

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Date palm (*Phoenix dactylifera* L.) is one of the most important tree crops in desert areas of Africa, Asia and the Middle East. It has a range of uses, providing fruit, materials for shelter, timber products and fuel. Date palm fruit is a stable and dependable food as well as a good source of carbohydrates, fibers, minerals and vitamins. Dates are known as having anti-mutagenic and anti-carcinogenic properties. In Sudan, date palm is a very important crop and it is grown in an area of about 36 204 ha.

In this study, the extent and structure of genetic diversity of date palm was explored through morphological characterization of mature trees on farms and molecular (SSR) analyses of collected samples. The morphological characterization was conducted on 116 date palm female farmers' varieties and 20 male farmers' varieties in the districts River Nile and Northern States. Eighteen vegetative and fruit characteristics (quantitative and qualitative) were evaluated. Ten SSR loci were used to characterize molecular diversity among 105 varieties out of 116 (92 female and 13 male). The investigated plant materials appeared to be very diverse in morphological characters. High level of morphological diversity was confirmed with SSR markers.

To investigate the current status of existing on-farm date palm production regarding preferred varieties and to assess threats facing the date palm culture, 215 date palm farmers were interviewed in River Nile and Northern State. The results indicated that the most preferred of the grown farmers' varieties was Barakawi. The main problems facing date palm cultivation in the Northern region of Sudan are introduction of new varieties, novel diseases and some socio-economic factors. The results of this study will contribute to the formulation of a national strategy for the conservation and sustainable use of date palm genetic resources in Sudan.

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# Genetic variability in *N. tabacum* as revealed by morphological traits and molecular markers

poster p1-15

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Tobacco (Nicotiana tabacum L.) is an allopolyploid species (n=24) in the large family of the Solanaceae and is one of the most economically important non-food crops cultivated worldwide. Numerous types of tobacco are grown and are classified, to a large extend, by region or area of production, method of curing and use in manufacturing, as well as by some distinct morphological characters and chemical differences. A tremendous amount of phenotypic variability exists among strains of *N. tabacum*, but polymorphism revealed by DNA molecular markers is comparatively low. At the present time only limited information is available on the relationship between morphological variability and genetic diversity at sequence level in N. tabacum. The aim of this study was to investigate on the genetic variability of tobacco genotypes as revealed by morphological traits and molecular markers (ISSR and SSR). In this study 50 tobacco genotypes, belonging to CRA-CAT germplasm collection of *Nicotiana* genus, were described by means of 4 quantitative traits and 18 multistate traits referring to plant habit, leaf, flowers and capsule morphology. Genomic DNA of the same tobacco genotypes were amplified utilizing 11 Inter-Simple Sequence Repeat (ISSR) primers and 35 Simple Sequence Repeats (SSR) primers. As regards ISSR markers, among the tobacco genotypes a total of 93 bands were produced of which only 11 (12%) were polymorphic. The size of ISSR fragments generated ranged from about 200 to 1900 bp. SSR markers produced from 1 to five bands each, with sizes ranging from 100 to 300 bp. About 50% of the SSR markers utilized showed polymorphic bands among the tobacco genotypes examined. Genetic variability revealed by morphological traits and molecular marker analysis will be discussed.

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## Molecular evaluation of tomato (Lycopersicon esculentum Mill.) genotypes using microsatellite markers

poster p1-16

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Tomato (Lycopersicon esculentum Mill.) is one of the most studied vegetable species in fields of breeding, genetics and genomics. It is a diploid species with 24 chromosomes, and a genome size of 950 Mbp which is composed of 77% heterochromatin and 23% euchromatin (1). Tomato belongs to the nightshade family *Solanaceae* and is considered to be one of the most important and world's traded vegetable. A great phenotypic variation in the genus Lycopersicon owns to its highly variable natural habitat. Human activities, started from domestication, and followed by artificial selection and creation of modern cultivars caused significant reduction of tomato genetic diversity. Taking into account very narrow genetic base of the most widely grown cultivars, significance of divergent initial material in breeding programs is remarkable. The objective of this research was to assess genetic diversity using microsatellite markers in 30 tomato genotypes which included wild relatives, local populations and old cultivars originating from different parts of the world. The analyzed material represents a part of a tomato collection of the Institute of Field and Vegetable Crops in Novi Sad. The SSR markers were selected from publicly available data and fragment size of PCR products was obtained by capillary electrophoresis using Applied Biosystems 3130 Genetic Analyzer. Cluster analysis was performed to classify the genotypes into groups. Data were processed in the statistical software Statistica 9 (StatSoft Inc. Corporation, Tulsa, USA). The results obtained are preliminary data for further association analysis and prerequisite for marker assisted selection in tomato breeding.

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## Studies on biodiversity of local onion (*Allium cepa* L.) landraces cultivated in saline areas from western romania through phenotipic and molecular analizes

poster p1-18

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The transformation of soils into saline ones is a frequent phenomenon in Romania and also worldwide, due to the accumulation of highly soluble salts at the surface or in the soil profile. The area covered by saline soils in Romania is 609,6 thousand ha, which represent 6,5 % of arable area.

The problems determined by salinity are associated mostly with other abiotic stress factors, as drought and phosphorus deficit. Quality improvement of these soils by using amendments associated with irrigation and drainage needs much too high investments for semi-subsistence agriculture, specific to Romanian rural areas. Other viable solutions, accessible to farmers are valuable local germplasm identification and breeding, which can be realized due to technological evolution and improvement of modern biochemical, physiological and molecular analytical instruments. Thus, the understanding and using of the genetic basis of cultivated species tolerant to salinity is possible.

Around 80 onion landraces adapted for saline soil conditions were collected and analyzed, from an area covering around 150.000 ha from Western part of Romania.

Phenotypic characterization were based on biometrical measurements on onion bulbs traits.

As a tool for genotype characterization and assessment of genetic diversity, DNA fingerprinting was used for all of the landraces.

ISSR (Inter Simple Sequence Repeat) markers were used, because it is a simple and quick method that combines most of the advantages of microsatellites (SSRs) and amplified fragment length polymorphism (AFLP) to the universality of random amplified polymorphic

DNA (RAPD). ISSR markers are highly polymorphic and are useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology.

The genetic similarity coefficients and dendogram, reflecting genetic relatedness of the landraces were established based on Inter simple sequence repeat (ISSR)-PCR fingerprint. Cluster analysis based on Jaccard's similarity coefficient using UPGMA grouped the landraces into different clusters, which were correlated with the phenotypic data.

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2 Food security

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### Screening, testing and molecular diagnosis for resistance against phyllody disease in sesame

poster p2-01

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Phytoplasmas are unculturable, wall-less prokaryotes and cause to phyllody in sesame. This disease inhabits most sesame-growing regions worldwide and it causes to stunting of the plants and alteration of the floral parts into leafy structures bearing no capsule and seeds, resulting in significant economic losses. Similar affects were observed in southern regions of Turkey and visual overall inspections of sesame fields indicated a disease incidence up to 50% (Ikten et al. 2011). In order to identify phyllody resistance source(s), a large scale sesame germplasm was investigated. Beside, the disease symptomatology, etiology, and also transmission in sesame were revealed. The field screening method for the identification of resistance to phyllody disease was applied amongst germplasm and breeding lines of sesame. Resistant, susceptible and lately infected plants were determined in this stage and plants scored according to resistance levels. After scoring, transmission studies (graft inoculation, and insect vector transmission) were conducted under controlled greenhouse conditions. The causative agent was successfully transmitted to healthy plants, producing disease symptoms within 25-35 days. Molecular diagnosis methods were also conducted to identify disease affected plants, possible vectors and agents. Based on molecular studies, different phytoplasma strains were observed and 16Sr-IX group phytoplasmas were reported for the first time in sesame (Catal et al. 2013). Resistant sesame accessions identified under field conditions are currently further investigations. These accessions are being confirmed using the vector insect and graft inoculation methods under greenhouse conditions.

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## Introducing host plant resistance to seed-borne diseases anthracnose (*Colletotricum lindemuthianum*) and *bean common mosaic virus* (BCMV) in Kyrgyz common bean through marker-aided and inoculation-based backcrossing

poster p2-02

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Common bean (Phaseolus vulgaris L.) is an important export crop in Kyrgyzstan. Bean common mosaic virus (BCMV) and anthracnose are seed-borne diseases that affect this crop and reduce its yield significantly in the country. The aim of this study was to identify virus strain(s) occurring in Kyrgyzstan and breed host plant resistance to BCMV and anthracnose though sequenced characterized amplified region (SCAR) markers and inoculation-based backcrossing. The use of DNA-based markers in backcrossing may help to speeding up the breeding for host plant resistance. Backcross methods were successfully used in this breeding program to improve Kyrgyz cultivars. Susceptible recurrent parents Kyrgyz cultivars Ryabaya, Kytayanka and Lopatka were included in a backcrossing breeding scheme for introducing host plant resistance from two donor cultivars namely Vaillant and Flagrano. After 4<sup>th</sup> backcross seeds (color, shape, size) and pods (shape) were as recurrent parents. The virus strains were evaluated according to the symptoms of differential cultivars. The virus strain NL6 was predominantly found in northern Kyrgyzstan, where farmers grow most of the common bean produced in this country. Two SCAR markers (SW13 and SBD5) were used successfully in marker-aided backcrossing for pyramiding the I and  $bc-I^2$  genes, which provide host plant resistance to BCMV. SCAreoli marker was as well used in backcrossing for transfer Co-2 gene into Kyrgyz susceptible common bean material. Combination of inoculation tests (anthracnose races delta and gamma, and virus strain NL3) and DNA-marker verify the resistance in progeny. BCMV resistant  $BC_4F_2$ , offspring carrying the I gene showed necrosis hypersensitivity after detached leaf-assays. Anthracnose resistant  $BC_4F_2$  offspring carry the Co-2 gene was without diseases symptoms. Resistance to seed borne diseases (BCMV and anthracnose) were combined into Lopatka line and BC<sub>4</sub>F<sub>2</sub> seeds will useful in future breeding program to improve Kyrgyz common beans.

Keywords: Common bean, BCMV, anthracnose

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# Evaluation of genetic diversity among open-pollinated sugar beet populations using quantitative root traits

poster p2-03

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The reliable information about genetic diversity in the breeding material is the basic requirement for development of successful breeding program and future crop improvement. Genetic diversity can be detected by pedigree analysis, by using morphological, physiological, cytological, or DNA markers, as well as biometric analysis of quantitative traits. The aim of this research was evaluation of genetic diversity among eight sugar beet open-pollinated multigerm populations from three different breeding programs using quantitative root traits. Two populations, EL0204 and EL53, were developed by USDA-ARS centers in East Lansing, MI and Salinas, CA. Both populations were released as a germplasm source for use in development of parental lines, primarily pollinators, combining smooth root and disease resistance to rhizomania (EL0204) and Rhizoctonia crown and root rot resistance (EL53, McGrath, 2006). Populations FC220 and FC221 were developed by USDA-ARS centers at Fort Collins, CO and Salinas, CA. Both had resistance to Rhizoctonia root rot and are segregating for the Rz1 gene, which confers resistance to some strains of Beet necrotic yellow vein virus, the causal agent of rhizomania (Panella et al. 2008). The four populations NS1, NS2, NS3, NS4, were from Institute of field and vegetable crops, Novi Sad, sugar beet breeding program. They were resistant to rhizomania, moderately resistant to Cercospora leaf spot, and adapted to local environmental conditions. Root weight, sugar content and crystal sugar yield per root were measured. The analysis of variance revealed significant differences (p = 0.05) among pollinators for all investigated traits. Cluster analysis was based on the data of all quantitative traits which were used in the research. On Euclidean distance around 1 there were identified three cluster groups. In the first group were populations EL0204 and EL53. The second group was the largest and it included populations NS1, NS2 NS3 NS4 and FC220. Population FC221 formed separate group and was joined with other populations at the highest hierarchical level. The results showed that breeding material from USDA-ARS centers is genetically diverse from NS populations and can be used for crossing and creating new genetic variability, and thus for providing wide recombination of genes in future offspring.

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## Association between heterosis and genetic distance based on morphological traits and SSR markers in *Cicer* species

poster p2-04

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Association between heterosis and genetic distance based on morphological and molecular data has been widely studied in vegetables and in many economically important plant species. Results have suggested that molecular divergence has been a good predictor for hybrid performance. The objectives of this study were: (i) to estimate the heterosis and heterobeltiosis of hybrids for yield and yield components in Cicer species; (ii) to measure the genetic distance among C. arietinum, C. echinospermum and C. reticulatum based on morphological traits and SSR markers, and (iii) to investigate correlations between heterosis of hybrids and genetic distances of the parents. Three Cicer species including two accessions (AWC 603 and AWC 613) of C. reticulatum, one accession (AWC 304) of C. echinospermum and three accessions of the cultivated chickpea (C. arieitnum), two accessions (CA 2969 and Mexican white) of 'macrosperma' and one accession (ICC 4969) of 'microsperma' chickpea were crossed. Genetic distance among Cicer species was detected using SSR markers and morphological traits. A significant correlation between the genetic distance of Cicer species and heterosis of the hybrid for yield and yield components was found. The performance of the hybrid for yield and yield components was higher in intraspecific crosses than those of interspecific crosses. Results also showed that morphological variation in F<sub>2</sub> generation was found to be higher in intraspecific crosses than those of interspecific ones. To obtain better hybrids and transgrasive segregations in the later generation, heterosis prediction would allow for reduction of time, cost and effort.

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## Correlations between *Rht*8/*Ppd*-D1 alleles and agronomic traits in winter wheat germplasm

poster p2-05

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Bread wheat is widely grown and productive cereal crop thank to polyploidy nature, crop plasticity and breeders ability to select the plants that can grow, flower and mature in a diverse agro-climatic environments. With population increasing, cultivated land decreasing, shortening of water supply and global climatic changes, more efficient utilization of precipitation and irrigation water resources, improvement of drought resistance and yield potential are the ultimate goals in wheat breeding today. Reduction in plant height from 150 to 70 cm is itself directly responsible for increase in productivity and resistance to lodging. The close linkage of dwarfing gene Rht8 and the photoperiodic insensitivity gene Ppd-D1 on chromosome 2D had a substantial role in determining the geographic adaptation in modern wheat varieties as well as their phenotypic expression traits (Worland et al. 1998). A total of 40 winter bread wheat varieties, originated from Croatia, Austria, France, Italy and Russia, were used in this study. The genotypes were sown in the Eastern part of Croatia using randomized block design with three repetitions. During growth season, harvesting and threshing, following traits were measured: plant height, heading date, 1000 kernel weight, number of days to heading and grain yield. Allele distributions of Rht8 dwarfing gene were detected by PCR analysis using microsatellite gwm261 according to Röder et al. (1998), while detection of Ppd-D1 alleles was conducted according to Beals et al. (2007). Discriminant analysis was carried out to perceive the differences of six Rht8/Ppd-D1 groups on the mean value of individual variables and to determine which of these variables was the most discriminant between groups. During canonical and correlation analysis the first two discriminant axis explained 83.9% of total variability between groups. The First canonical axis showed the highest difference between groups 165/b and 192/a, while the second axis mostly discriminate groups 196/b and 174/a. Based on discriminant analysis results distances between centroids of every groups were calculated using the Mahalonobis coefficient. Coefficients between groups varied from 2.1\* to 26.8\*\* and it was possible to discriminate groups according to some agronomic traits.

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## Prickly lettuce - enormous source of variation unexploited in lettuce breeding

poster p2-06

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Prickly lettuce (Lactuca serriola L.), weedy plant species and close wild relative to cultivated Lactuca sativa L. is used in lettuce breeding as an important donor of valuable traits (e.g. resistance) since twenties (Lebeda et al., 2007). Accessions in world germplasm collections cover only a limited area of its world round distribution. Data on L. serriola natural distribution and biodiversity are missing (Lebeda et al., 2009a) as well as those on prickly lettuce variation. Research activities of authors include eco-geographical studies and collecting, regeneration protocols, morphological characterization, evaluation of response to downy- and powdery- mildews and study of AFLP polymorphism. Original data on ecology and distribution of L. serriola in Europe, North America and Near East were obtained, new seed samples were acquired for further studies. According to the relative DNA content L. serriola is grouped with L. dregeana, L. angustana, L. perennis (Lebeda et al., 2007). The composition of sesquiterpene lactones in leaves is similar to those in L. sativa and L. dregeana (Lebeda et al., 2009a). Large phenotypical and phenological variation among samples related to their geographic origin was recognized (Lebeda et al., 2007, 2009a). Enormous variation in the reaction patterns to downy- and powdery mildews was recognized and new sources of resistance to economically important races of both mildews (Lebeda et al., 2008). Samples originating from various eco-geographical conditions (Near East vs. Mediterranean Basin) differ significantly in their polymorphism (AFLP) and they are genetically different (Lebeda et al., 2009b). L. serriola was crossed with L. sativa, and F1 hybrids were fully fertile.

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## Assessment of resistance to apple scab (*Venturia inaequalis*) of apple genetic resourcesand breeding applications at the Institute of Horticulture, LRCAF

poster p2-07

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A qualitative and quantitative resistance to apple scab (*Venturia inaequalis*) is characteristic to *Malus* sp. plants, therefore identification of resistance genes and efficient introduction of the genes to apple cultivars is crucial for apple breeding. Current horticultural research incorporates application of genetic and biotechnological approaches for identification of new sources of resistance to apple scab (Gessler et al., 2006; Kellerhals et al., 2009). In addition, the importance of pyramidization of resistance genes is emphasized in breeding programmes due to the genetic variability of the scab pathogen and the risk of loss of resistance in widely grown apple cultivars.

Monogenic Vf resistance remains effective protection against apple scab in part of the Northern Europe. Previously, three apple cultivars 'Skaistis', 'Rudenis' and 'Aldas' with Vf resistance were released from the Institute of Horticulture, LRCAF. Another two apple accessions featuring Vf resistance traits were developed during last decade. Further breeding activities included pyramidization of monogenic resistance by combination of *Vf*, *Vm*, *Va* genes. PCR based molecular markers were used to identify genotypes carrying complex monogenic resistance traits during early development stage of seedling obtained from crosses involving cultivars such as 'Orlovim', 'Reglindis', 'Rubinola' and hybrids Nr.24087, 'Freedom' x 'Murray', 'Noris' x 'Antonovka', 'Priam' x 'SR0523'.

Apple trees have been grown in the Northern Europe for centuries, and a number of traditional cultivars carrying genes for qualitative and quantitative resistance are spread in the region. However, pedigree of many of the traditional cultivars is unknown, and information on genetic background of resistance of the cultivars is scarce. A number of accessions of the traditional cultivars and cultivars derived from crosses with the traditional cultivars are deposited at the collection of genetic resources of the Institute of Horticulture, LRCAF. A study on genetic polymorphism and morphological traits of 37 cultivars revealed 13 genotypes as potential sources for apple scab resistance breeding. Further, a biotechnological method of screening for apple scab resistance at embryonic stage using apple seed cotyledons was employed to resolve a complexity of genetic background of resistance of the identified genotypes.

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### A novel reduced culm number mutant in rice

poster p2-08

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Seedling vigor is one of the most important breeding objectives for stable and high productivity under cool temperature condition in the areas of high latitude or high altitude regions in rice. However, very few genes have been cloned to date resulting that molecular basis of seedling vigor under cool temperature condition is still unclear. Therefore, it is important to clone and elucidate the molecular function of new seedling vigor genes. Our previous reports represented that an ABC transporter, designated as Rcn1/OsABCG5, is an essential transporter for shoot and root development (Yasuno et al., 2009; Ureshi et al., 2012). Here we identified a novel reduced culm number mutant, designated as reduced culm number1-like (rcn1-like), which represented a reduced shoot development involving main shoot and tillering and root development under cool temperature condition in Hokkaido, the most northern area of Japan. Allelism test demonstrated that rcn1-like was independent rcn1 on rice chromosome 3. Molecular mapping study represented that *rcn1-like* located on rice chromosome 8. *Rcn1* was up-regulated in *rcn1* shoot and root showing that *Rcn1* is controlled by the negative feedback regulation. In *rcn1-like*, no effect on the amount of *Rcn1* expression was observed. The present genetic and molecular characterizations of rcn1-like demonstrated that a novel mutant is independent of Rcn1/OsABCG5 associated transport system and will facilitate the understanding of a new mechanism for seedling vigor under cool temperature condition.

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## Allelic diversity of genes regulating growth habit and photoperiod response in temperate cereals maintained at the VIR germplasm collection

poster p2-09

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Among the key traits that have been involved in wheat and barley domestication was adaptation of flowering time to different areas. Migration of the cereal crops to regions outside its place of origin was accelerated through mutations in genes regulating growth habit (*Vrn* genes) and photoperiod response (*Ppd* genes). Allelic diversity of the genes currently maintained in genebanks is a valuable source for breeders to develop new cultivars with improved adaptive properties to address the issue of climate change.

The combination of *Vrn* and *Ppd* alleles has significant influence on the development pattern of wheat and barley cultivars. An association between developmental rate and biomass accumulation was reported, showing that later flowering genotypes grew slowly, whilst earlier flowering genotypes showed significant variation in biomass production. Gene-specific markers currently available for *Vrn* and *Ppd* in wheat and barley allow breeders to select the allele combinations that would be optimal for specific conditions of cultivation.

We employed the molecular marker assay to assess the vernalization and photoperiod response characteristic of 490 advanced wheat and barley cultivars included in Recommended List Varieties for Russia 2012. Restricted allelic variation was detected in the cereal crops currently recommended for commercial propagation in Russia. Only 3% of wheat and 9% of barley spring varieties carry dominant alleles of *Ppd* genes. The severe deficiency of dominant alleles of *Vrn* and *Ppd* genes in the domestic cereal germplasm could be replenished by employing new donors of the beneficial alleles of the adaptation-responsive genes.

Among the new sources of the valuable alleles there is a photoperiod insensitive wheat breeding lines carrying a new *Ppd-B1a* allele. So far the main source of photoperiod insensitivity in soft wheat was the dominant *Ppd-D1a* allele on chromosome 2D. Near isogenic lines developed at VIR have recessive alleles in *Ppd-D1* and *Ppd-A1* loci, but have different photoperiod sensitivity due to the polymorphism in *Ppd-B1* locus confirmed by SSR-genotyping and allele-specific PCR assay. Another valuable sources represent wheat varieties carrying the rare dominant allele in *Vrn-B3* locus. The allele is associated with the retrotransposon insertion in the *TaFT* gene promoter. This mutation has not yet been used extensively in commercial varieties and, therefore, represents a potentially valuable source of genetic diversity to modulate wheat flowering time.

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## Phenotypic variation of a *Triticum monococcum* L. core collection

poster p2-10

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The good nutritional properties of einkorn (*Triticum monococcum* L.) suggest that this ancient wheat is a promising candidate for the manufacturing of new and special foods. The einkorn collection maintained at CRA-SCV (> 1600 accessions, domesticated and wild) represents a valuable pool of genetic variability, harbouring useful alleles for disease resistance, production and quality to be used in intraspecific and interspecific crosses within plant breeding programmes. To best utilise this broad genetic variability, an association mapping project was started in 2010. To this end, a core collection including 169 accessions representative of the variation observed in the whole collection and selected following geographic and qualitative criteria, was assembled. The core collection was then characterised both phenotipically (two locations, two years) and genetically (microsatellites and AFLPs).

The variation observed among einkorn accessions was relevant for all the main traits scored (heading time, plant height, ear length, n° of spikelets per spike and of seeds per spikelet, 1000 kernels weight, proteins and carotenoids content). When the geographic origin of the accessions was considered, the ANOVA showed the existence of significant differences between samples of different origin for most traits. In general, heading was later for einkorns from continental Europe than for those from Mediterranean countries. On the contrary, the kernel weight of southern Europe samples was higher than that of northern regions. Einkorns from different countries were significantly different for spike length, but no clear south-north gradient was observed; however, the number of spikelets per spike increased from south to north. Other traits with significant differences among samples from different origin were seed length, protein content and the bread-making quality related trait SDS sedimentation volume.

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## Shifting trends of bread wheat breeding in Italy assessed by the study of a temporal-based core collection

poster p2-11

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Germplasm collections represent valuable genetic resources for breeding programmes which aim to the synthesis of improved genotypes, better suited to the technological and nutritional needs of the market. One of the more recent ways to exploit the natural genetic diversity of germplasm collections is the so-called association analysis, or association mapping (AM), whose basic objective is to detect correlations between genotypes and phenotypes on the basis of linkage disequilibrium (LD). The sheer size of many germplasm collections, however, often hinders their best exploitation; hence the need to identify core collections, i.e. representative manageable samples where to perform the initial assessments, before re-exploring broader ranging materials.

Given these assumptions, within the huge *Triticum aestivum* collection available at CRA-SCV, a sub-collection of 157 lines was identified and an association mapping project was started. The genotypes, selected following a temporal principle (year of constitution or diffusion) integrated with production and quality characteristics, summarize the process of wheat breeding in Italy during the latest hundred years.

The lines were cropped for two growing seasons in two different locations in Italy and assessed for several agronomic, morphologic and qualitative traits. In parallel, DNA was extracted and analysed by the 90K wheat Infinium array (Illumina). The phenotypic traits were analyzed by an univariate (*i.e.*, ANOVA) and some multivariate approaches (*i.e.*, MANOVA, Correspondence analysis and modeling). Both approaches showed not only significant differences for all the parameters studied, but also could describe the association between phenotypic variables; a clear trend from old to new varieties was observed, leading towards earliness, plant size reduction, kernel weight decrease, kernel hardness increase and protein content reduction (coupled, nevertheless, with an improvement in protein quality). The statistical analysis for the definition of allele diversity, the phenomic analyses (*i.e.*, coupling genotypes and phenotypes information), population structure and linkage disequilibrium is currently under way.

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## Evaluation of apple and pear genetic resources in Bosnia-Herzegovina with the aim of sustainable, commercial utilization

poster p2-12

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In order to assess traditional apple and pear germplasm in Bosnia and Herzegovina (B&H), accessions from both *ex situ* collection and *on farm* locations have been investigated using microsatellite markers (Gasi et al., 2010; 2013). Molecular data obtained, revealed a high level of genetic diversity inside the examined germplasm and a significant genetic differentiation between traditional apple and pear cultivars, compared to international cultivars of these two fruit crops, currently cultivated in Bosnia and Herzegovina. Although the morphologic traits registered among traditional apple and pear accessions in Bosnia and Herzegovina are very diverse (Gasi et al., 2011), this does not necessarily mean that most of the genetic material is appropriate for traditional breeding purposes. However, specific chemical composition of the traditional (B&H) apple and pear fruit might make them suitable as a raw material for processing into products such as pekmez, jam, cloudy juice and brandy, using both traditional and modern methods. In order to evaluate apple and pear genetic resources in Bosnia and Herzegovina for these purposes we are currently investigated chemical content of the fruit as well as sensory characteristics. Some of the key preliminary results are presented here.

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## Barley landraces as a potential of disease resistance in Nordic barley breeding

poster p2-13

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Plant production is challenged by several pressure factors. Effects of biotic and abiotic stresses are increasing and production demands requirements for low-input and minimal environmental impact. Plant breeding is a tool to be prepared for tomorrow. Disease resistance based on plant genes is an environmentally friendly way to manage plant pathogens. Genetic resources, landraces and wild types, may carry the needed traits to survive in future conditions and to minimize cultivation risks. To benefit on the plant genetic diversity the potential value can be channeled to modern varieties through pre-breeding.

In this research, potential sources for barley leaf spot disease resistance were studied in diverse barley cultivars and landraces with different historical and geographical origin. The main objective was to compare level of resistance between barley cultivars and landraces against four common leaf spot diseases in Finland: net and spot types of barley net blotch (*Pyrenophora teres* f. *teres* and *Pyrenophora teres* f. *maculata*), scald (*Rhynchosporium secalis*) and spot blotch (*Cochliobolus sativus*). Also, the aim was to find potential resistance sources for future barley breeding programs to produce new variation with adaptation to climate change. The study showed that high levels of resistance against leaf spot diseases already exist among improved European barley cultivars originating from different countries in comparison to the majority of the studied Nordic barley landraces. However, landrace genotypes may contain some unused sources of resistance that can be employed to improve the existing barley varieties and make resistance more durable. Wide range of useful resistance was found among Syrian and Jordanian barley landraces.

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### Barley and oats are new naked crops, aren't they?

poster p2-14

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Naked barley and oats are new food and forage crops. Naked barley belongs to the species *Hordeum vulgare* L. and is represented by spring cultivars, mostly two- and sometimes six-row ones (Lukyanova et al., 1990). Besides, six-rowed cultivars of winter barley have been developed recently. Naked forms of barley have such valuable characters as high content of protein, lysine, oil, sterols, tocopherols, etc. Some accessions of spring naked barley have been analyzed for  $\beta$ -glucans content. The content of said components in two-row barleys was shown to be on the average 1.5 times higher than in six-row barleys. The grain of naked barleys (two- and six-row forms) was found to be much more resistant to *Fusarium* blight than that of hulled forms (Gagkaeva et al., 2011).

At present, there exists some confusion concerning naked oats. Initially, C. Linnaeus (1762) designated naked diploid oat as *Avena nuda* L. Later on, they started applying the name *A. nuda* L. to the naked forms of cultivated oats, while the diploid forms were given the name of *A. nudibrevis* Vav. (Vavilov, 1927). B. Baum (1977) restored the initial name *A. nuda* L. for the diploid naked forms, but the hexaploid naked forms were not determined in this taxonomy. Therefore, in the classification of oats by Rodionova et al. (1994) these naked forms were assigned the rank of the hulled forms subspecies: *A. strigosa* subsp. *nudibrevis* (Vav.) Rod. et Sold. and *A. sativa* subsp. *nudisativa* (Husn.) Rod. et Sold. Besides, the once lost naked forms of *A. byzantina* C. Koch., that is *A. byzantina* subsp. *denudate* (Hausskn.) Rod. et Sold., have been rediscovered (Loskutov, Rines, 2011).

The results of comparative studies of the naked and hulled forms have shown that many of their agronomic characters are substantially different. In terms of biochemical composition, a grains of the naked form significantly higher from that of the hulled form by a content of protein, amino acid compositions, oil, fatty acid compositions and antioxidants (sterols and tocopherols) (Loskutov, 2007). The grain of naked forms has been found to be much more resistant to *Fusarium* blight than that of hulled forms (Gagkaeva et al., 2013).

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### The effect of added barley chromosomes on agronomic parameters of wheat in greenhouse

poster p2-15

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Hybridization between related species makes it possible to transfer useful traits such as stress tolerance, earliness and other desirable traits from one species into another. The 2H, 3H, 4H, 6HS and 7H addition lines of winter wheat 'Martonvásári 9 kr1'/winter barley 'Igri' produced in Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences in Martonvásár were investigated to determine how the added barley chromosome influences agronomic traits, especially yield components of wheat.

The addition and parental lines were grown in 1,5 l pots in greenhouse in Martonvásár. All the pots received weight irrigation until 60% field water capacity. Data were obtained for anthesis- and maturity dates, plant height, fresh and dry weight of root, number of spikes, ear length, thousand grain weight, number of kernels, and grain yield.

The highest root-shoot ratio was measured in case of 4H addition line. The introgression lines exhibited marked difference in anthesis- and maturity dates, two weeks difference was observed between 7H and 4H addition lines, 7H being the earlier. The 3H addition line was the shortest and the 2H line the tallest among the examined lines. The spike-length was found to be proportional to plant height. The thousand grain weight of 7H addition line was the highest, although this line had a poorer grain number per spike and low grain yield per pot.

We measured a scale of variation in all examined traits. The introgression lines originating from wheat-barley hybrids can broaden the genetic bases of the wheat breeding programs with valuable traits.

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## The Maize Germplasm Bank at CIMMYT : An Invaluable Genetic Resource for Maize Geneticists, Breeders, Producers and Consumers throughout the World

poster p2-16

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The Maize Germplasm Bank (MGB) at CIMMYT (The International Maize and Wheat Improvement Center) in Texcoco, Mexico, holds the world's largest collection of maize germplasm, currently numbering over 27,000 seed accessions, in a subterranean, earthquake-proof, temperature- and humidity-controlled vault. Although the CIMMYT germplasm bank has been distributing seed of landraces, improved materials and CIMMYT inbred lines (CMLs) to researchers, breeders and farmers for nearly 50 years, only recently has the seed request process been available to the public via the CIMMYT website (www.cimmyt.org/obtainseed). In addition, the MGB database will be uploaded to the new GRIN-Global system in 2013, and for the first time will be publically accessible online. In order to achieve and maintain the highest quality seed for our global clientele, the CIMMYT Maize and Wheat Germplasm Banks jointly achieved ISO9001:2008 certification in December 2012. We are only the third germplasm bank in the world (and the first outside of Europe) to achieve certification. In addition to the maize collection, the MGB holds over 200 seed accessions of the closest wild relatives of maize, the "teosintes" (Zea mays sensu lato and other Zea species), as well as a live germplasm collection of the perennial sister genus to Zea, the genus Tripsacum. The MGB has been providing landrace germplasm for the GEM (Genetic Enhancement of Maize) Project and for ongoing genotypic and phenotypic evaluation by the CIMMYT-MasAgro "Seeds of Discovery" (SeeD) Project, and is currently conducting targeted phenotypic surveys of the germplasm collection, also in collaboration with SeeD. All of these efforts are part of the MGB's mandate to preserve in perpetuity the genetic diversity of maize and to facilitate the use of these genetic resources to meet the challenges of achieving food security for the world's growing population.

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## On the novel variation generated in the phytomeric structure of barley spike

poster p2-18

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Barley is one of the most important cereal crops which ranks fourth in terms of production and cultivation all over the world. It is a model organism for genetic and genomic studies in Triticeae, due to the high degree of natural variation and wide adaptability, the diploid genome structure and the strongly inbreeding-based mating system, as well as the wide array of genetic and genomic resources. Wide genetic variations for developmental traits have been observed in barley and this peculiarity is believed one of the main evolutionary driving force for its broad adaptability. Collections of morphological barley mutants are available (Pozzi et al., 2000; Forster et al., 2007; Stanca et al., 2013), which include stem, leaf, ear, flower, awn and grain mutants, as well as double and triple mutants obtained by intercrossing simple mutants, and near isogenic lines (NILs) useful for deep genetic analyses. Our attention in this work is mainly focused on the morphological mutants of barley spike, which affect the phytomeric structure of the rachis (long/short internode, branched), florets (two-rowed/six-rowed, multiflorus, reduced lateral spikelets, deficiens, laxatum), lemma (leafy lemma), glumes (third outer glumes, wider outer glumes), awns (awnless, short awns, triple awned, long awned glumes, branched long awned glumes, calcaroides, Hooded, elevated hooded, seeded in hood), grains (colorless/colored grain, naked/hulled grain, waxy). A new Hooded mutant has been observed in rare outcrosses of the extra flower in the Hood, named "Seeded in Hood", in which small seed in some Hood is developed. This mutant generated a complex segregant population from which several Hooded double mutants have been isolated. Genetic studies are in progress to define the segregation of the F2 derived by the cross "Seeded in Hood" x Cometa (two-rowed commercial variety). Most of this mutants, behind the morphological and genetical studies, will provide breeders with original material for pre-breeding work.

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## Characterization of tomatoes landraces preserved on USAMVB collection

poster p2-19

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The key of tomatoes landraces utilization in breeding consists in a complete features description of collected genotypes. The tomatoes collection preserved on USAMVB was subject of ex situ prebreeding evaluation. In order to point out the valuable peculiarities of 31 landraces, it was used IPGRI Descriptors for Tomato. The aims of our work were the following: yield and its quality. The experiment was organized in field and in hydroponics. Mass and individual selection pointed out an unexpected diversity. Plant characteristics: the plant growth was of indeterminate type (68.03%). determinate (9.6%), and mixed (16.14%). Plant size was large (>150 cm - 29.13%) and normal (38.63%). Native dwarf landrace were not found. Valcele-2 plants were taller than 2.5 m being proper to select very tall plants for protected long-term culture. Coseni-1971 spitted in dwarf and indeterminate plant's growth (36±2.6 and 138±3.3cm respectively). The agronomic characteristics: it has been noted an early and tardy flowering (55 and 73 days respectively). The fruits' maturity varied from 110 (Conop-p3) to 124 (Seitin-2Y) days respectively. In the first quarter of ontogenesis R-25 and R-27 performed 31.6% and 32.0% respectively of yield. At the dwarf and short genotypes the 50% of yielding capacity was ready in the first half part of the growth. The yielding hierarchical arrangement revealed plants/elite and landraces proper for a "single" harvest. The plant 5 of R-25 and plant 1 of R-27 performed 65% and 50% of yield in the first quarter. The yield quality was evaluated using the BRIX index, ascorbic acid amount the antioxidant capacity (FRAP) and content of metals. BRIX index varied in large limits on Coseni-1137 (0.8 to 2.2). It was high on Conop plants (CN-1287; 2.4 - 4.3). At CN 1137 the ascorbic acid content and FRAP were 23 µM ac.galic/100 g product and 169-µMFe/100 g product. Content of K was 200x higher as normal (22.9 mg/100g WM. No heavy metals as Co, Cr were detected; in low content 0.01 ppm was lead. The drying processing technique was tested. In comparison with the majority landraces Coseni-318, Porodici and L-62 are suitable for drying in room condition.

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### Evaluation, characterisation and utilisation of Latvian pea (*Pisum sativum* L.) genetic resources

poster p2-20

### Agnese Gailite<sup>1</sup>, Skaidrite Bumane<sup>2</sup>, Anita Gaile<sup>1</sup>, Dainis Rungis<sup>1</sup>

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Increasing emphasis is being placed on the need for northern Europe to become more self-sufficient in the production of protein crops. This necessitates increased utilisation of legumes, including peas. Grey peas are a staple food in Latvia, and have been traditionally grown and used for food. There is still a strong consumer interest in grey peas, particularly at Christmas and New Year celebrations. However, the area under cultivation is quite small, and the vast majority of production is utilised for local consumption. All the varieties grown are Latvian cultivars, developed at the State Priekuli Plant Breeding Institute. An application for geographical designation for one of the local cultivars has been made in collaboration with a local farmer and a distributor. The Latvian gene bank holds a total of 63 pea accessions, the majority of which are grey peas. The collection includes 10 varieties, 1 landrace, and 52 accessions which were repatriated from the N.I.Vavilov Research Institute of Plant Industry (VIR) collection. In some cases, the identity of these repatriated accessions can be deduced from the names, but the identity of the majority of them is unknown, and may include landraces, breeding lines and other germplasm. Descriptors for peas have been developed, and all 10 varieties have been characterised. In addition, SSR markers have been utilised to genetically fingerprint the majority of the collection. The SSR data has been analysed in an attempt to identify the genetic relationships of the unknown accessions repatriated from the VIR collection with the accessions with known provenance. Genetic diversity within the collection was also assessed.

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## Conservation of the agricultural and horticultural plants - Ten years of the National Plant Genetic Resources Programme in Finland

poster p2-21

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The Finnish National Plant Genetic Resources Programme was founded in 2003 to meet the goals of the international agreements (CBD, IT). MTT Agrifood Research Finland is responsible for the coordination and conservation of the agricultural and horticultural crops. Programme is monitored by National Advisory Board for Genetic Resources appointed by the Ministry of Agriculture and Forestry. Work concentrates mainly on the vegetatively propagated plants, while seed samples are stored in the Nordic Genetic Resource Center (NordGen) in Alnarp. National collections are located as plants in the field genebanks around Finland, in in vitro -preservation, and/or in cryopreservation at liquid nitrogen tanks. Cryopreservation is beneficial particularly for vegetatively propagated plants to ensure long term storage, and protocols are in use with some berry species. Decisions of the mandates and organization of the collections are made in the national working groups. Preferred is diverse material from various origins, well adapted to our conditions like long cold winters, short growing season with long day conditions, and often acidic soils. Plant inventories have been made to find landraces and local types still available and missing from our collections. Much of this work is carried out in different projects, like recent calls for fruit trees, potato onions, hops and cereals. Diversity of the collected material is analyzed by DNA-markers if possible, to select genetically wide material and discard duplicates. Fingerprinting is done especially in fruit and berry plants, and we are looking forward to include other plant groups as well. Information of vegetative collections is managed nationally, and the data of accession are to be transferred to SESTO data management system. Securing stable funding for activities is a challenging task. In addition to the basic conservation work, funds should be found for evaluation of the collected accessions for different traits, in order to enhance their use for plant breeding purposes, or for developing new landrace-based products. Knowledge of the cultural history aspect may serve as attraction for increasing tourists' interest for local gardens and parks, or enhance home-gardeners interest to buy ornamentals originated for the locally adapted genetic resources.

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# Evaluation of genetic variability for FHB resistance in oat germplasm

poster p2-22

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A wide oat germplasm collection has been evaluated for genetic variability in resistance to *Fusari-um* spp. and to toxin accumulation. Screening of oat genotypes was carried out under conditions of artificial inoculation or after natural *Fusarium* infestation. Wide variability for susceptibility/resistance has been found among oat genotypes belonging both to the primary and secondary gene pools. A set of qPCR based assays was applied to the identification and quantification of different fungal species that have been involved in mycotoxin productions in the oat samples. The correlations between quantity of fungal DNA evaluated with these assays and quantity of mycotoxins have been determined.

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## Protocols for Phenotyping Segregating Maize Gene Bank Accessions

poster p2-23

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Maize Landraces stored in gene banks are populations with a high level of heterogeneity and heterozygosity between and within them. Maize is naturally open pollinizated and farmer selection has over millennium has produced groupings for specific morphological, environmental and use characteristics that are identifiable as races of maize. To adequately characterize such heterogeneous materials it is necessary to evaluate the levels of segregation within accessions for the traits evaluated. Traits such as male and female flowering dates, plant and ear heights, disease resistance, number of ears, are not adequately measured by measuring a single plant or by the row median. Data must be taken on an individual plant basis with the number of individual plants measured being sufficient to capture the segregation within the accession for the trait desired, thus providing a measurement of the variability between plants in the same accession. With this in mind, new protocols were developed to obtain precise and accurate data from a plant basis that allows calculation of internal variability. The Seeds of Discovery Initiative, whose goal is to open the bottle neck between maize and wheat germplasm banks and breeding programs, has genotyped a single plant per accession of the 4000+ accessions in the CIMMYT Genebank's Maize Breeder's Core Collection. The single plant genotyped per accession was crossed onto 10 plants of a CIMMYT hybrid of the same environmental adaptation to produce an accession/testcross for phenotypic characterization. Segregation is still evident within these modified testcrosses due to the heterozygosity of the accession parent. We have in several trials taken data both, by plot and by individual plant. The measured traits were days to silk and anthesis and fusarium. Trials were designed as augmented row column design with two or four replicated checks. Data was analyzed by a linear mixed model including the intra-plot, between plants effect. Overall results are similar in both approachs, by plant or by plot. Take data by plant is more time consuming than in a plot basis, but that is the only way in which we can effectively estimate the variability intra accession.

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## The evaluation of genetic diversity of agronomical traits in a part of gene bank of snap bean

poster p2-24

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To evaluation the agronomic and morphologiocal charecters and classify a part of gene bank snap bean collection, and determine the rellationshipudy between germplasm a total of 64 genotypes samples planted in 2010 at bojnourd branch Islamic azad university in simple lattice design (8\*8) with two replication . The result showed that Sunray variety had the highest yield. Ther were corrolatins among betwem yield and biological yield, number of seed, pod number. Stepwise regression showed that the total pod had the most important trait. Path analysis indicated that selection must be done on biological yield. Factor analysis showed that traits were summarized in five factors, and this factors justified 75.5% of the total variation of data. All genotype classified in four different groups. In order clasasify these 64 cultivars, they were clusterd by UPGMA and WARD methods. The resulting dendograms showed different groups involving cultivars wrer formed.

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## *Ex situ* evaluation of grain yield in local landraces of faba bean (*Vicia faba* L.) from Serbia

poster p2-25

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Faba bean (Vicia faba L.) originated in Central Asian centre of diversity. Faba bean is one of the most ancient cultivated plant species in the world, along with several other grain legumes, and cereals. Numerous archaeobotanical findings, supported by rich linguistic evidence (1), witness its early and rapid distribution during Neolithic from Near East to Europe, North Africa and Central and East Asia and an important role it had in everyday diets of the ancestors of modern Old World nations. Today, faba bean is one of the most important annual legume crops for human consumption, animal feeding and for non-food purposes. Conservation and sustainable utilisation of faba bean genetic resources play the most significant role in contemporary faba bean breeding programmes and developing novel faba bean cultivars with high and stable grain yield, enhanced quality aspects and improved resistance to both abiotic and biotic stresses (2). Serbia is an example of a country where faba bean used to be cultivated to a great extent until few centuries ago, when it was gradually and in the end almost fully replaced by common beans (Phaseolus vulgaris L.). Today, faba bean has survived at several locations in Bačka and Srem, in the north, and especially in the areas adjacent to the valley of the river South Morava, in central and southeast parts of the country (3). Since 2009, expeditions have been made to local green markets and households of these regions, aiming at collecting locally grown and maintained faba bean landraces. A small-plot trial has been carried out during 2011 and 2012 at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi, including 30 local landraces of faba bean from several villages in the vicinity of Kruševac. The two-year average highest grain yield was in the population Gaglovo 3 (4537 kg ha<sup>-1</sup>), while the highest two-year average crude protein content was in the population Pepeljevac (284 g kg<sup>-1</sup>). Further research will comprise the evaluation of tannin contents and other nutritional aspects.

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# Characterization of Estonian field pea (*Pisum sativum* L.) genetic resources

poster p2-26

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Field pea breeding has been conducted in Estonia for more than 90 years. During this period 12 varieties have been released at Jõgeva Plant Breeding Institute (PBI). Besides these varieties, four landraces and 105 breeding lines of Estonian origin and 73 foreign accessions of field pea are maintained at the Genebank of Jõgeva PBI.

During the years 2010-2012, field trials were carried out at Jõgeva PBI on characterization and evaluation of 14 field pea varieties and landraces.

Accessions were examined for 32 descriptors in accordance with pea genetic resources evaluation methodology (Peas..., 2006).

Morphology of foliage and inflorecences were characterized and disease (*Ascohyta pisi, Erysiphe pisi, Perenospora pisi, Uromyces* ssp.) resistance was estimated during the growing period; characteristics of pods and seeds were determined at full ripening stage. Yields of the seeds and protein were calculated on the basis of dry matter and nitrogen contents. All studied accessions were of leafed type and with high (>25%) protein content.

Landraces were characterized by tall ( $\geq$ 160 cm) stems and good disease resistance. Plant appearance was distinctive in a landrace 'Kollasekirju söödahernes' with its light green foliage, and variety 'Jõgeva kirju' with anthocyanin ring on its leaf armpit.

Green cotyledons were characteristic to six accessions, while yellows were observed in eight. Three accessions had anthocyanin coloration on wing, 11 were with white flowers.

According to the results, disease resistant landraces of pea with high nutritional value are suitable for further breeding for fresh forage.

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## Nordic spring barley - diversity of agronomic traits and molecular markers

poster p2-27

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Spring barley is an important crop in Nordic countries. Currently, both two-rowed and six-rowed barleys are grown. Historically six-rowed types have been grown here longer - since third and forth millennia B.C., whereas two-rowed barley has been cultivated here only since the early to mid 17<sup>th</sup> century (or in some parts since the 18<sup>th</sup> century). Nowadays, six-rowed cultivars are still common in Norway and Finland due to the good adaptation to more northern conditions, but in Sweden and Denmark only two-rowed cultivars are present. Barley breeding has been carried out in Nordic countries for more than a century, resulting in a great number of improved cultivars. The majority of the cultivars released over these years as well as research and breeding material are maintained and available at the Nordic Genetic Resource Center (NordGen), where barley has the largest share in the whole collection. The availability of this material has allowed several research studies, including evaluation of historic changes of barley genetic diversity in the region.

Significant qualitative and quantitative changes of diversity in Nordic spring barley have been demonstrated using phenotypic and genetic markers as well as changes in agronomic performance. The differences of diversity changes in two-rowed and six-rowed material have also been shown. This paper aims to give an overview of the results of diversity studies on Nordic spring barley in order to illustrate the breeding effect on diversity.

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## New sources of resistance against net blotch in barley landrace collection

poster p2-28

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*Pyrenophora teres* Drechs *f. teres*. Smedeg (Ptt), the cause of net blotch, which is one of the most widely distributed and destructive foliar diseases of barley (*Hordeum vulgare* L. emend. Bowden) especially in cool and humid regions. Net blotch is considered as economically important because can cause reduction in both yield (up to 40%) and crop quality (kernel size and weight, malt extract). The yield components affected by net blotch depend on the time of disease onset and the severity and duration of the disease.

Strong environmental pressure to reduce pesticides causes new requirements in plant breeding programs. The breeding of suitable cereal varieties with high and stable yield, adequate quality traits and low environment impact will be dependent on the available appropriate genetic sources and the subsequent selection and trialing for adaptation to agronomic, policy and market requirements.

Genetic variability present in landraces was not fully employed in modern barley breeding programs. The purpose of this study was identify new sources of resistance in 212 lines selected from ICARDA barley landrace collection.

Experiment was conducted in 2009 and 2010 in Poland and Finland (IHAR-PIB and MTT). Three independent experiments were conducted on seedlings in the greenhouse (IHAR, MTT) and in the field (MTT).

In first experiment (IHAR), conducted on detached-leaves, 212 barley lines were inoculated with 15 Ptt isolates with different virulence against net blotch. These isolates were collected from field in Radzików. In second experiment (MTT) the same set of cultivars and isolates were tested in the greenhouse on seedlings. In third experiment conducted in the field (MTT) 212 lines were inoculated with Ptt isolates mixture.

This study shows the presence of great variability among landraces regarding resistance to net blotch. New sources of resistance to net blotch were found in tested lines. These new lines may contribute significantly to diversity of net blotch resistance gene pool available for barley breeders.

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## Barley landraces in pre-breeding programmes for diseases resistance

poster p2-29

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Powdery mildew and leaf rust, caused by *Blumeria graminis* DC. f.sp. *hordei* Marschal and *Puccinia hordei* Otth. respectively, are prevalent foliar diseases of barley (*Hordeum vulgare* L.) in Europe. Yield reduction as high as 40% and reduced yield and grain quality occurred when the diseases were served. Development of resistant cultivars is the most economical and environmentally safe measure to control the diseases. Many studies showed that barley landraces are rich in genetic diversity with respect to disease resistance. Some of the resistance genes have been introgressed into elite germplasm. However, barley breeders are constantly looking for new efficient sources of resistance to order to increase its durability.

This study aimed in identifying of effective resources of resistance to powdery mildew and leaf rust in barley landraces for pre-breeding purposes. It was conducted in the frame of Pre-breeding national project financed by Polish Ministry of Agriculture and Rural Development: Determine the interaction between biotic stresses resistance and features of the economic value of spring barley No.: HOR hn-801-8/12.

Five elite barley cultivars (Conchita, KWS Alicjana, Victoriana, Rubinek and KWS Olof) with Mlo resistance were crossed with four selected landrace lines characterized by resistance to leaf rust (isolate Rubinales with virulence against *Rph7*) and powdery mildew. In 2012, families F3BC1 derived from crosses of these lines and cultivars were tested in the seedling stage for resistance under controlled conditions. In total 5705 lines were inoculated by powdery mildew and leaf rust isolates avirulent, respectively to *mlo* and *Rph7* resistance genes. For further study 248 plants resistant to both pathogens were selected. Introduction of the new effective resistance genes to elite barley cultivars for pre-breeding purpose requires further studies the these new germplasm lines in different environmental conditions.

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## Variation in susceptibility to *wheat dwarf virus* among wild and domesticated wheat

poster p2-30

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Bread wheat (*Triticum aestivum*) is a crop species with a hexaploid genome (2n = 6x = AABBDD), originating from a natural hybridization between the wild diploid *Aegilops tauschii* (D) and the tetraploid wild emmer wheat, *Triticum turgidum* ssp. *dicoccon* (AB). In general, domesticated plants have experienced vast phenotypic and genetic changes as a result of domestication and loss of resistance in bread wheat has been described.

In this study, we focused on the response to *Wheat dwarf virus* (WDV) and we hypothesize that the susceptibility of bread wheat to WDV is a result of the loss of resistance during domestication. Eighteen wild and cultivated wheat species (genera *Aegilops, Amblyopyrum* and *Triticum*) were evaluated for traits associated with resistance to WDV. The plants were inoculated with WDV in a greenhouse experiment using leafhoppers (*Psammotettix alienus*) as vectors. The eighteen species evaluated displayed a large variation in the morphological traits after WDV inoculation. The WDV-infected plants all displayed reduction in height, shoot dry weight, number of tillers and number of leaves as well as increased chlorosis and increased mortality. However, the range of severity in symptoms varied greatly and the response also varied over time for some species. Different response patterns were found among the species and some wild relatives of wheat performed better over time than others. Other wild relatives were found to be highly susceptible. Serological analyses (DAS-ELISA) showed increased virus levels in the domesticated wheat group in comparison to the wild relatives of wheat. Two-way ANOVA analyses showed that the WDV inoculation had a significant effect on all traits studied.

Upon these findings we have continued to characterize the virus-host interaction for the progenitor species of the A genome (*Triticum urartu*) and the D genome (*Aegilops tauschii*) during infection of WDV.

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# Long-term conservation of plant genetic resources in Lithuania

poster p2-31

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A National Plant Genetic Resources (PGR) Coordinating Centre was established at the Institute of Agriculture in 1993 for coordination activities on PGR investigation and preservation. The Baltic - Nordic PGR project was initiated by Nordic Genetic Resource Center (former Nordic Gene Bank) in 1994. It promoted eight Lithuanian research institutes to consolidate their efforts to start development of the national PGR conservation system. The Government of the Republic of Lithuania enacted the decision to establish Plant gene bank since the 1st of January 2004. Its main functions are to co-ordinate the collection, research, conservation and use of the national plant genetic resources in Lithuania and keep the genetic material in long-term storage. Eight institutions are involved in the PGR network. Institutions are responsible for collection, research and conservation of plant genetic resources according to different plant groups (agricultural crops, horticultural plants, forest trees, medical and aromatic plants and ornamental plants). Plant Gene Bank is responsible for long-term storage of PGR and 2686 accessions are preserved. The longterm seed storage is annually supplemented by new accessions. Accessions representing 158 plant species. Old landraces and varieties of agricultural crops, advanced varieties and valuable breeding material, as well as distinguished populations of wild relatives of cultivated plants and forest trees have already been stored in the long-term seed storage at Plant Gene Bank. Germination test of 1940 seed accessions was carried out and was ascertained that percentage of geminated seeds of 268 accessions was at lest 15% lower than it was determined before seeds were put into long-term storage.

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### Plant Genetic Resources for Food and Agriculture in Kazakhstan, the State of *In-Situ* and On-Farm Management

poster p2-32

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Kazakhstan is a country rich in primary and secondary gene pools of many cultivated and wild plants, but there have been complete changes of many ecosystems, as a result of the large-scale scarification of the land (1954-1960). During the last few years, a number of species are no longer found within the territory of Kazakhstan. The Convention of Biodiversity (CBD) was signed by Kazakhstan, along with 152 countries in (1992). In order for the ratification of the CBD, three national scientific programs have been accepted for the conservation and sustainable use of PGRFA. In this paper a brief description of the state of *in-situ* management in the Republic of Kazakhstan is presented.

Plant genetic resources are a major factor in steady agricultural production; the genetic resources and play an important role in the preservation of the ecological equilibrium in the environment. Additionally, special importance for the maintenance of food safety has been noted in the last decade by a number of international documents (CBD, 1992; GPA, 1996; and ITPGRFA, 2004). Kazakhstan is a region rich in both primary and secondary gene pools of many cultivated and wild plants (VAVILOV 1930). According to PAVLOV (1956), the Kazak flora consists of 5,700 species, which according to ABDULLINA (1999) out of 6000 species that are vascular plants, 210 species are crop wild relatives. In Kazakhstan, there have been deep changes to many ecosystems; deep anthropogenic transformation of the steppe and of the forest-steppe zones, as a result of large-scale scarifying of lands during 1954 - 1960.

According to the "National Report on biodiversity" (1998), during the last few years, a number of species can no longer be found in the territory of Kazakhstan. Seventy plant species are included in the first edition of the Kazakhstan Red Data Book, and are protected by the Government. These materials are being prepared for the second edition of the Red Data Book of Kazakhstan, which will include 404 species of plants. According to the"National program of action with regard to the desertification in the Republic of Kazakhstan" (Almaty, 1997), half of the 19 species of compound forage plants are threatened with extinction in the near future. Crop wild relatives, and wild plants for food, are under direct threats of genetic erosion (BAYTENOV 1984, BAYTENOV 1986). Landraces, farmers' varieties, unique genetic materials; as well as genetic sources of high resistance to diseases and to pests, cold, and drought are either completely lost or destroyed (URAZALIEV 1992, URAZALIEV 1995). The Convention of Biodiversity (CBD) was signed by Kazakhstan in 1992. For ratification of the CBD, three national scientific programs have been accepted for the conservation and sustainable use of PGRFA, which have designated the 4 basic directions of research: collection; evolution; documentation and storage of agricultural plants' germplasm, which were valuable attributes selected as sources and economic donors. First, the national programs on genetic resources of agricultural plants are directed toward the identification, particularly of genetic diversity, and the threats to that genetic diversity in *ex-situ* collections. The conservation of biodiversity in situ/ex situ is a priority for the national program on plant genetic resources.

Key words: plant genetic resources, in situ management, on farm conservation

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## Biological diversity and cultivation potential of yellow nutsedge in East Africa

poster p2-33

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Parts of East Africa suffer from food and nutrition insecurity mainly because of reliance on a few staple food crops. For this reason, there is need to diversify food sources by adopting non-conventional crops. Tuber crops such as cassava, sweet potato and yams are valuable food security crops in Africa during periods when cereal supplies are limited. Yellow nutsedge (Cyperus esculentus) is a widely distributed tuber producing species that grows as a weed in Eastern Africa, but under cultivation in several countries. Nutsedge tubers have high nutritive value as a source of protein, carbohydrates, edible oil, fibre and a variety of minerals. However, there is limited knowledge on the extent of diversity of yellow nutsedge, although the cultivated and wild forms show substantial variability. In this project we assess the potential of yellow nutsedge as a crop for East Africa by determining the amount of genetic diversity, yield and composition of tubers of nutsedge accessions collected from different localities in East Africa. Thirty three (33) wild nutsedge accessions from Kenya, Uganda and Tanzania and 5 cultivated types commercially grown in Egypt or Spain were evaluated. The thirty eight (38) accessions varied in morphology, and in the yield size and colour of tubers. Using sequence information derived from the transcriptome of developing tubers of one cultivated type, 89 putative simple sequence repeat (SSR) markers were identified. Of these, 20 polymorphic markers were the most reliable for studying genetic diversity of the yellow nutsedge accessions. This is the first attempt to develop and use SSR markers for yellow nutsedge genetic diversity studies. Tubers harvested from thirty five (35) accessions raised under controlled growth conditions in a climate chamber were analysed for oil content and fatty acid composition. There was significant variation in both the oil content (6.57 - 28.23 mg/100mg of dry tuber) and fatty acid composition among the accessions. Two wild accessions had higher oil content than some of the cultivars. These results indicate that there is great potential for nutsedge improvement through breeding. The fatty acid profile was similar for both the wild and the cultivated types, with oleic acids contributing about 70 %, and palmitic and linoleic acids each making up 10 % of the total fatty acid. Based on results on the nutritive and economic potential, field trials have been set up in Kenya to evaluate selected nutsedge clones for agronomic characteristics.

Keywords: *Cyperus esculentus*, Genetic diversity, food security, tuber yield, nutrient composition, oil content.

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3 Nutrition and health: Genetic diversity for health and nutrition

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## Recent achievements and current status of *Vitis vinifera* germplasm in South East Europe

poster p3-02

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The viticulture of South East Europe is to the some extent "personalized" due to large number of autochthonous (unique) varieties, but from end of 19th century first mildews and Phylloxera plagued and reduced diversity in vineyards, and this process was continued through globalization of wine industry (Cipriani et al., 2010). Ampelographic and molecular characterization of national germplasm collections and local varieties has been done in many European countries, but in countries of South East Europe, grapevine material is still not properly genetically evaluated and compared due to fragmented projects and approaches. Even if the SSR genotyping was done, results were not standardized, stored and compared with international databases, so origin of some material is still unknown/ misunderstood. Well managed genetic grapevine resources provide basic material for selection and improvement trough breeding, but also can provide a starting point for revitalization of rare varieties. Recently, several independent projects of regional collaboration for grapevine germplasm analysis made a thorough inventory of native South East Europe germplasm (Žulj Mihaljević et al., 2013). Aim of this study was to give an overview of the current status of Vitis vinifera germplasm in neighboring countries of South East Europe through survey of recent literature and projects of regional collaboration. Recent achievements in SSR genotyping of some supposedly autochthonous and unknown accessions will be presented through their comparison with internal microsatellite database, the European Vitis Database and publications in order to determine synonyms and/or homonyms. Twenty-one synonyms and possible synonyms are presented. Their trueness to type and their distribution across countries is discussed, as well as recommendations for further ampelographic expertise.

Key words: grapevine germplasm, South East Europe, microsatellites, synonyms

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## Genetic diversity for mineral contents in some Common bean landraces (*Phaseolus vulgaris* L.)

poster p3-03

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In Kosovo, Common bean (*Phaseolus vulgaris* L) is grown in intercropping system with maize or as single crop in a small scale in more intensive farming system. Planting area with common beans are about 7.505 ha, with an average of yield 0.9 t ha<sup>-1</sup>, while the annual consumption per capita is 11.53 kg. The long tradition of common bean cultivation in Kosovo has allowed the evolution of many landraces adapted to restricted rural areas.

A set of 10 landraces were collected from different sites across the Country, in different localities. Little information exists about mineral content and other quality traits for those bean genotypes. The study was performed in order to assess the chemical content for 7 different minerals of common beans landraces originating from different localities in Kosovo.

The aim of this research was to evaluate the genetic diversity for grain mineral content among different common bean landraces, landraces belonging to Gene Bank of Kosovo (GBK); the content of 4 macronutrient (K, Ca, Mg and Na) and 3 micronutrient (Cu, Fe, and Zn), important in human nutrition. The factorial experiment was set up as randomized complete block design (RCBD) with three replications. Statistical calculations were performed using ANOVA and LSD-test and software Minitab-16.

Average contents of various macronutrient and micronutrient minerals in dry grain for different common bean landraces were:  $K-\mu=1414.07 \text{mg kg}^{-1}$ ,  $Ca-\mu=176.2 \text{mg kg}^{-1}$ ,  $Mg-\mu=116.98 \text{mg kg}^{-1}$ ,  $Na-\mu=20.6 \text{ mg kg}^{-1}$ ,  $Cu-\mu=1.244 \text{ mg kg}^{-1}$ ,  $Fe-\mu=5.4 \text{ mg kg}^{-1}$  and  $Zn-\mu=2.65 \text{ mg kg}^{-1}$ .

Results show a significant difference (LSDp=0.05 and 0.01) among landraces for mineral content (Cu, Ca, Na, K, Fe and Mg), but for Zn content there is no significant difference.

The Pearson's correlation coefficient among traits generally showed high positive significant values for (Na, K, Mg /Ca and Mg/K) and negative value for (Na/Cu and Fe/Zn).

The identification of landraces with high content of minerals, increase the value of landraces. This information could be useful to determine their quality and to promote its consumption. The genetic diversity among landraces makes them a valuable resource as potential donors of genes for breeding and development of new cultivars of common bean.

Key words: Common bean, landraces, diversity, mineral content, genetic resources

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## Determination of grain quality traits and some chemical properties in the whole grain of spring barley germplasm

poster p3-04

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Nowadays a strong focus is placed on the high quality of food and input materials. The barley grains could be used as an important source of fibre and mineral substances. The aim of the present study was to investigate grain quality traits and some chemical properties of spring barley cultivars and breeding lines and to compare the concentrations of macro- and microelements in the whole grain of spring barley. A total of 20 samples of spring barley grain were analyzed for protein, starch, dietary fibre, fat, ash contents and concentrations of P, K Mg, Na, Fe, Cu and Zn. During the period 2011-2012, the spring barley genotypes showed variability of protein content from 10.5 to 16.4 %, starch content - 57.1-64.8 %, dietary fibre content - 3.11-6.94 %, fat content - 1.16-2.21 % and ash content - 1.76-3.11%. The concentrations of minerals in barley grain samples varied as follows: P from 2955 to 5370 mg kg<sup>-1</sup>, K from 4020 to 7630 mg kg<sup>-1</sup>, Ca from 130 to 600 mg kg<sup>-1</sup>, Mg from 630 to 1890 mg kg<sup>-1</sup>, Na from 420 to 880 mg kg<sup>-1</sup>, Fe from 40.38 to 68.68 mg kg<sup>-1</sup>, Cu from 3.01 to 4.92 mg kg<sup>-1</sup>, and Zn from 16.84 to 29.28 mg kg<sup>-1</sup>. In most cases, there were significant positive correlations among the concentrations of Fe, Zn, Cu, P, Mg. Negative differences were obtained between Mg and Ca. Total ash, P and Cu contents significantly positively correlated with protein content (P < 0.05). Comparison of the two experimental years showed that year as a factor had the greatest influence on fat and Ca content, lesser on starch content. Significantly lower year influence was exhibited on dietary fibre content.

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## Diversity of characteristics of dill poster p3-05

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Dill refers to potherbs. It is cultivated for fresh salad production and also as a raw material for drying, freezing and essential oil extraction.

Dill is popular in Russia – more than 50 varieties are known, though it is insufficiently studied as an object of breeding. First of all, diversity of characteristics and their inheritance are poorly investigated.

Descriptions of dill varieties include attributes characterizing habit of seeds, plants, leaves, floriferous shoots, inflorescences and flowers. Varieties are also distinguished by time of appearance of floriferous shoots.

Let's examine some of the characteristics.

According to the shape of rosette of leaves the following types of dill plants are distinguished:

- elevated rosette - leaves grow at an angle 45 and more degrees to the surface of soil;

- smooth rosette - leaves grow almost in parallel to the surface of soil, at an angle 5-10 degrees;

- semi-elevated rosette - leaves grow at an angle 5-45 degrees to the surface of soil.

Most of commercial varieties of dill have semi-elevated rosette of leaves. Smooth rosette of leaves is characteristic of varieties Salut and Buyan.

Varieties Amazon, Almaz, Iney, Alligator, Anker have elevated rosette of leaves. Leaves of variety Anker grow almost vertically - at the angle about 60-70 degrees to the surface of soil.

Interesting characteristic of variety Preobrazhensky was revealed recently – short hypoctyl and internodes in comparison with other dill varieties. Other varieties have mesocotyl which become drawn and seedlings fall down at high temperatures.

Plants of variety Preobrazhensky are always compact and dwarf.

Time of appearance of floriferous shoots in conditions of long day (not less than 14 hours) is an important economic characteristic.

There are plants with early appearance of floriferous shoots – in 45-50 days after germination. Varieties Gribovsky, Karusel, Preobrazhensky can be relegated to this group.

The second group includes plants with medium term of floriferous shoots appearance – 51-60 days after germination. This is characteristic of varieties Kutuzovsky, Kibray, Iney, Anker.

The latest appearance of floriferous shoots (in 61-90 days after germination) was observed in varieties Amazon, Alligator, Salut, Buyan, Almaz. This last group is the most interesting for getting greens due to longer period of production income and gross yield in comparison with more early ripening varieties. But seed production of this group of varieties is more complicated and more expansive due to longer vegetative period.

An interesting characteristic of variety is presence and intensity of wax coating on the leaves. Strong wax coating is typical for almost all varieties of dill. This feature is useful for vegetation of plants, but decreases market quality of products. Lack of wax coating or its minor display together with glossy shiny surface of leaves significantly improve appearance of commercial yield. These characteristics are present in variety Rannee Chudo (Early Wonder).

An important characteristic of dill leaf is shape, size and density of the ending segments location. They can be filamentous (varieties Kibray, Ambrella) and flattened (varieties Alligator, Preobrazhensky). Depending on density of segments location varieties with rarer (Richelieu) and thicker (Iney) density can be distinguished. The other varieties have medium density of leaf segments location. Sevastopolsky variety is characterized by wider leaf segments. Short segments were observed in varieties Amazon, Alligator, Preobrazhensky, longer segments - in varieties Richelieu and Anker. In future it is expected to examine the principles of inheritance of the above characteristics in hybridization.

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## World Olive Germplasm of Córdoba (Spain) as a source of variability for olive selection and breeding

poster p3-07

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The richness of the cultivated olive germplasm represents an unusual case among horticultural crops, as a consequence of tree longevity and lack of turnover with new breeding genotypes. More than 1,200 varieties are still under cultivation, 79 international and national collections located in 24 countries. Among them the World Olive Germplasm Bank (WOGB) of IFAPA, Centre "Alameda del Obispo", Córdoba (Spain) is the most important olive collection at both national and international scale. It includes 885 different accessions from 24 different countries. The identification of the olive cultivars, an important and ongoing task, has been carried out by means of both traditional and molecular (SSR, DArT and SNP) markers. The combined information obtained by these markers has made possible the identification of the WOGB revealing synonymies (different names for the same cultivar), homonymies (the same naming for different cultivars) and propagation errors. Besides, the WOGB has been subject of continuous evaluation of agronomic (tree characters, fruit traits, oil content, pest and disease resistance) and olive oil quality characteristics. The results obtained by both agronomic evaluations and the above mentioned molecular markers confirm that the germplasm collection is a useful source of genetically diverse material for researchers, olive breeders and growers. The most interesting cultivars are being evaluated in comparative trials at both national and regional level in order to select the best ones for different growing conditions. Additionally, the variability found at WOGB has been continuously used for selection of genitors in breeding programs (Rallo et al., 2008), which has allowed wide ranges of variation for selection of new cultivars. The joint use of both agronomic and molecular data has also made possible the development of the first core collection in olive (Belaj et al., 2012). This core collection included 36 cultivars, representative of Eastern, Central and Western Mediterranean area, representing more than 90% of the diversity found in the whole collection.

Keywords: Olea europaea, genetic resources, cultivars, genetic improvement

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## Association mapping for content of phytic acid and Pi in wheat germplasm

poster p3-08

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Phytic acid (PA, InsP<sub>6</sub>) is the main storage form of phosphorus in cereal seeds. PA is considered an anti-nutritional factor as it is a strong chelator of iron and other minerals, reducing their bioavailability. This phenomenon may contribute to "hidden hunger" in human populations where cereals are the primary source of nutrition. In husbandry the main problem is that monogastric animals cannot digest and utilize PA, which might result in environmental phosphate pollution due to excretion of PA. Reduced content of PA in the grain is thus a target for molecular breeding of wheat in order to improve quality of the grain for both food and feed.

Association mapping is a method to identify significant associations between markers and traits of interest in large collections of e.g. wheat varieties, and can be used to dissect complex quantitative traits and identify candidate genes for the trait(s) of interest. In the present study we have evaluated a collection of 169 hexaploid wheat varieties grown under ecological conditions at Agrologica, Denmark as part of the BIOBREED project<sup>1</sup>.Content of phytic acid phosphorous (PA-P) and Pi in the grain was quantified using colorimetric assays and were found to range from 3,9-5,4 mg PA-P/g seed and 0,2-1,26 mg Pi/g seed, respectively. Neither PA-P nor Pi content was correlated to grain weight. These phenotypic data will be combined with genotypic data from approximately 2500 polymorphic DArT (Diversity Arrays Technology) markers distributed over the wheat genome to identify markers associated with the two traits. This may increase our understanding on the genetic regulation of PA and Pi content in the wheat grain and may in the future lead to development of markers useful for breeding.

1 http://molbreed.life.ku.dk:8080/biobreed

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## Nutrient content in wheat and the NAM-B1 gene – A story of domestication and crop improvement

poster p3-09

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The transition of domestic crop species from wild progenitor to high-yielding modern varieties is characterized by two major events, domestication and crop improvement, both involving the introduction of new and strong selection pressures acting on the species. One suggested domestication gene is *NAM-B1*(previously Gpc-B1) in wheat. The gene has antagonistic effects with the wildtype allele giving faster maturation time and higher protein and mineral content in the seed. Two null alleles instead lead to slower maturation time causing larger yield, but with a lower nutrient content. The wildtype allele, first discovered in wild emmer wheat, has obtained much interest from plant breeders for its ability to increase protein content in durum and bread wheat.

Although originally suggested to be a domestication gene we could show, using historical collections of 19<sup>th</sup> century seeds that the wildtype allele was still present in widely cultivated bread and spelt wheats during the latter half of the 19<sup>th</sup> century. Screening a genebank maintained core collection covering world-wide bread wheat diversity pointed to environmental components being important in preserving the wildtype allele in bread wheats. In particular, landraces and obsolete cultivars from the Nordic region were found to possess the wildtype allele and could be exploited a genetic resource for protein increase in wheat breeding.

To further elucidate the evolutionary history of *NAM-B1*, we sequenced a number of fragments in the genomic region of *NAM-B1*. Sequence data from accessions of different tetraploid wheat species paints a picture the interacting roles of natural and strong artificial selection on *NAM-B1*.

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## Chasing valuable *Ribes* material in the Northern and Central European gene bank collections

poster p3-10

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Different fruit varieties are needed for different use. Both farmers, consumers, trade and the industry working on the production of upgraded products are nowadays increasingly interested in carefully selected fruit material. There is call for the varieties carrying fruit with high amounts of substances shown to be related to health-promoting effects. On the other hand, disease-resistant fruit varieties suitable for organic cultivation are demanded. Also fresh-fruit quality is important when consumers' preferences are considered.

In the EU AGRI GEN RES project "RIBESCO – Core Collection of Northern European Gene Pool of *Ribes*", the characterization data of over 1400 black, red and white currant and gooseberry accessions were collected. The evaluation work was based on the gene bank collections in some Northern European Countries, i.e. Finland, Estonia, Denmark, Latvia, Lithuania, Sweden, Poland and Germany. In addition to the extensively recorded morphological characteristics, also agronomic traits and some fruit quality aspects were evaluated.

The work on the collected data is continued in the EU funded FP7 project "EUBERRY – The sustainable improvement of European berry production, quality and nutritional value in a changing environment: Strawberries, Currants, Blackberries, Blueberries and Raspberries". Specific sets of varieties or strains are selected, to combine important traits for present use and for breeding especially in the Central and Northern Europe. The groups are designed to make up the sets of germplasm with a certain purpose. "The resistant group" includes varieties combining disease resistance characteristics and winter resistance. "The fresh fruit group" includes varieties combining for instance good fruit size, high sugar level and low acidity. The varieties in "The good health group" combine high vitamin C and high anthocyanin contents in the berries. The results of the work will be utilized in recommending varieties for cultivation as well as selecting material to breed new European *Ribes* varieties.

Key words: fruit quality, disease resistance, currants

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## Local landraces of maize in Serbia - A potential source for quality improvement

poster p3-12

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The natural diversity of maize has been significantly reduced during the decades of intensive maize breeding. Diverse local varieties are the valuable source of useful traits that could be used in breeding for abiotic and biotic stress resistance and quality improvement. The first maize flints varieties were introduced in Serbia in 18th century, whereas the US Corn Belt Dents were introduced later, at the end of 19th and the beginning of 20th century. After the introduction of dents, spontaneous crosses with more diverse flints increased their variability and adaptability. The Serbian maize gene bank currently comprises 5475 accessions, with 2217 landraces from former Yugoslavian countries which were collected in the expeditions from 60s of 20th century onwards. The classification and evaluation of variable local germplasm grouped it in 18 agroecological types (1). Landraces showed to have higher level of protein and amino acids content than commercial hybrids (2). Landraces belonging to flint types have higher protein content than dent type landraces. The highest values of protein were determined for varieties: Yellow trvdunka derived flints (16.86%), Eight-rowed maize type of Northeastern America Krizevac small-kernel (16.31%), White Moravac glassy (16.05%), Dents type of US Corn Belt dents Brdska zobanka (15.92%) and Serbian dents Kosmaj local white (14.24%). The highest values of lysine content have varieties: Yellow eight-rowed (489 mg/100g dry matter) and Derived flints types Jeremin rumena (509 mg/100g dry matter), Yellow ten-rowed (473 mg/100g dry matter). The highest values of tryptophan have White eight-rowed soft dents (65.6 mg/100g dry matter), White Moravac dent (62.2 mg/100g dry matter), Vrsac derived dent (63.2 mg/100g dry matter) and Guca glassy flinty dents (62.4 mg/100g dry matter). The autochthonous landraces are carriers of favourable alleles and can be used for improvement of the existing modern genotypes with a narrow genetic variability. Hence, the importance of maize local landraces is being revived in many regions of the world, especially in the light of principles of sustainable agriculture and the role local maize germplasm plays in areas with limited resources where human nutrition and livestock feed depend on the quality and ratio of amino acid content.

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## Nutritional value of Latvian Origin triticale, barley and potato

poster p3-13

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The aim of the study was to evaluate the influence of growing technology on yield quality traits of Latvian origin winter triticale, spring barley and potato. Field trials were arranged with four winter triticale, six spring barley and 12 potato genotypes under conventional and organic growing conditions at State Priekuli Plant Breeding Institute from 2010 to 2012.

For triticale, three different fertiliser rates were applied in the conventional field and two seeding rates were applied in the organic field. Quality traits of the yield as 1000 grain weight, protein and starch content, volume weight, falling number, as well as amount of amino acids were detected.

For barley, three different fertiliser rates were applied in the conventional field. Different seeding rates were applied for some barley genotypes under both growing conditions. Quality traits of the yield as 1000 grain weight, content of protein, starch and  $\beta$ -glycan, as well as amounts of Fe, Zn, tocopherols and amino acid lysine were detected.

For potatoes, two different fertiliser rates were used in the conventional field. A tuber yield and content of starch, as well as of vitamins A, B, and C in tubers were detected.

The study was performed with financial support of Latvian State Research Programme NATRES FOOD (2010–2013).

Key words: yield quality, triticale, barley, potatoes

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4 Crop wild relatives. Multifunctional agriculture, climate change

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## Facilitating the fishing in the gene pool of the wild eggplant relative *Solanum incanum* by developing introgression lines

poster p4-01

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Despite the economic importance of eggplant (Solanum melongena), breeders have made little use of the wild genepool, even though fully fertile hybrids are obtained with some of them (1). Solanum incanum is a wild close relative of eggplant naturally occurring in dry areas from northern Kenya to Pakistan (2). It presents resistance to drought, cold, and to some diseases, as well as high levels of bioactive phenolic compounds, in particular chlorogenic acid. However, S. incanum also presents many undesirable characteristics typical of wild species. Development of introgression lines (ILs) facilitate the use of genes from wild species by breeders (3). In order to develop a set of ILs of S. incanum in the genetic background of S. melongena we obtained the interspecific hybrid, which was highly fertile, very vigorous, and proved to be an excellent rootstock for eggplant. This interspecific hybrid was backcrossed with the S. melongena parent to obtain the BC1 generation. A genetic map in which markers were distributed in 12 linkage groups (LGs), which matches the haploid chromosome number of eggplant, was developed from the BC1 generation. The genetic map allowed the marker assisted selection in the BC1, and subsequently in the BC2 and BC3, of a set of plants that, while covering the whole genome of S. incanum, presented a reduced proportion of wild alleles in the rest of the genome. At present, the BC4 generation is being genotyped. The results obtained up to now show that with one or two additional BC generations followed by fixation by means of selfing, a set of 60-80 ILs (with five to eight ILs per LG) covering the whole S. incanum genome in the S. melongena genetic background, will be obtained. We have also been able to map seven genes involved in the chlorogenic acid (CGA) synthesis pathway, and a set of seven BC3 plants, each of which is carrying the S. incanum allele for a different CGA pathway gene, has been identified. The set of ILs will be of great utility for eggplant breeders and will allow the ready use of genes of interest from S. incanum in developing new eggplant cultivars with new characteristics and adapted to present and future needs (1).

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## Tuft morphological characterization of wild and cultivated Solanum species *in-vitro*conserved in Gene Bank

poster p4-02

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First evaluations of plants genetic resources were based in morphological traits. Tuft morphological characterization of wild and cultivated *Solanum* species according to EVIGEZ (Plant Genetic Resources Documentation in the Czech Republic) descriptor list in agro-ecological conditions of the Czech Republic was aim of this research. The two-year research included cultivated and wild *Solanum* species represented in 31 genotypes (*S. acaule 00030, S. andigenum 00108, S. berthaultii, 00260, S. brevidens 00240, S. bulbocastanum PIS 06-17, S. chacoense 00037, S. chacoense 00230, S. demissum 00250, S. fendleri 00275, S. goniocalyx 00109, S. gourlai 00045, S. gourlai 00043, S. guerreroense 00280, S. incamayoense 00047, S. leptophyes 00048, S. microdontum 00049, S. mochiquense 00050, S. phureja 00308, S. pinnatisectum 00051, S. polyadenium 00290, S. polytrichon 00053, S. sparsipillum 00071, S. spegazzini 00060, S. stenotomum 00212, S. stoloniferum 00295, S. sucrense 00062, S. vernei 00069, S. vernei 00234, S. verrucosum 00299, S. x chaucha 00134 and S. yungasense 00070)* 

Biological material was provided from potato *in vitro* gene bank, part of Potato Research Institute in Havlíčkův Brod. In the end of 2010, *in vitro* preserved genotypes were brought and assessed in Department of Genetics and Breeding (DGB). The genotypes from test tubes were propagated to the jars 0.1*l* with standard MS medium (Sigma). By ten plants of each genotype were prepared. Field experiment was completely randomized with two replications. Morphological characterization was done according to EVIGEZ descriptor list for following characteristics: Plant-tuft form, plant-tuft shape, plant-height, stem-erection, stem branching, stem thickness, stem colour, stem-number per plant, leaf shape, leaf pair number of primary leaflets, leaf shape of lateral leaflets, leaf leaflets presence, leaf lobation, leaf concrescence type of terminal, leaf surface, leaf size, leaf colour, leaf lustre, inflorescence position of pedical articulation, inflorescence anthocyanin colour of pedical articulation, inflorescence degree of flowering, inflorescence pollen fertility, inflorescence buds throwing off and berries number per plant.

High morphological diversity of tuft characteristics among the researched species was noticed. A cluster analysis was performed by Complete Linkage using Euclidean distances in software Statistica 9.1 Eng (StatSoft Inc.).

Key words: diversity, EVIGEZ descriptor, Solanum, tuft characterization

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## Evaluation of the diversity in cultivated lettuce (*Lactuca sativa* L.), contribution of wild accessions and old varieties

poster p4-03

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*Lactuca* L. genus belongs to *Asteraceae*, a large plant family, and is represented by several species distributed in different geographical areas and ecological conditions. Probably issued from a cross between *Lactuca serriola* L. and another wild species, lettuce (*Lactuca sativa* L.) is one of the most consumed fresh vegetables, with many morphological variations. The different morphotypes can be separated in two groups: the "not headed lettuce" with **Looseleaf** and **Chinese lettuce** and "lettuce which could form a head", with oblong head and thick leaves like *Romaines* (=Cos) and *Latines* or with circular head like **Butterhead** and two types of Batavian: **Summer crisp** and **Crisphead** (=Iceberg).

The French official organization for variety and seed testing (GEVES), carried out a study on the available diversity in cultivated lettuce species and its evolution.

This study on 500 lettuce varieties registered between 1952-2010, issued from the 6 morphotypes (except Chinese lettuce) present in Europe and characterized by 19 phenotypic traits and 30 SSR markers, shows that the structuring is principally based on morphotype and that since the middle of 80's the phenotypic and allelic diversities increase overtime. These diversities are uneven according to the observed cultigroups and introduction of new alleles is not uniform overtime. During last 25 years, progress has been made with introduction of novel traits including disease and insect resistances, novel leaf colors and shapes, open head, slow bolting or broad environmental adaptation.

New allele introduction is principally linked to the introgression of diseases resistances often realized by interspecific crossing: *Bremia lactucae* races, in constant emergence, still force breeders to find new sources from resistances in old varieties or wild species (*e.g. L. serriola* or *L. saligna...*); resistance to aphids (*Nasonovia ribisnigri*) is introgressed from *L. virosa* with *L. serriola* as bridge-species.

Nearly all enhancements have targeted traits from the primary gene pool especially for morphological traits in old varieties or populations. The secondary and tertiary pools have been recently used for diseases resistances traits.

In a context of sustainable intensification of global agriculture, the creation of varieties having more stable resistances goes through the genetics resources.

Key words: lettuce, diversity, segmentation, disease resistance

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## Genetic Resources and Ornamental Plant Breeding in Latvia

poster p4-04

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Latvia is situated in the vegetation zones between Northern and Central Europe. The country distinguishes itself with a large variety of flora - total approximation 1,800 species, of which 1,310 species are local vascular plants. Breeders are interested to find wild materials and use them in the production of new plants. With these wild resources, breeders not only develop new ornamental plants, but also bring the excellent properties from the wild materials into cultivars. The aim of the research was to evaluate the genetic resources with high ornamental value of genus Allium L., Lilium L., Tulipa L. and Gladiolus L. Chives - Allium shoenoprasum L. is rare and grows mostly in river valleys. Ramsons - Allium ursinum L. is rare and usually grows in monodominant groups in shady broad-leaved forests among shrubs. Over recent decades, under the impact of human activity the A. ursinum has decreased considerably – despite strict protection. Currently the Red Data book has played a certain role in the protection of endangered species. Martagon lily - Lilium martagon L., in Latvia was discovered in 1839 (Andrusaitis, 1985). It is rare, grows as solitarily specimens or in small groups - mostly in calcareous soils among shrubs, ravines and forests. Bulbous lily - Lilium bulbiferum L. is rare in the wild, but is widely cultivated. Wild tulip - Tulipa sylvestris L. is rare; naturalized ornamental plant that grows in groups in old parks and in park vicinities - in floodplain meadows by rivers. Shingled gladiolus - Gladiolus imbricatus L. is rather rare, grows in groups on floodplain meadows, on river banks and at the edges of forests. Although Latvia has a tradition of ornamental plants of more than 200 years, their breeding was started only after World War II. In the last 30 years, the new cultivars of lilies, gladiolus, dahlas and tulips have been obtained. The International Lily Register included 13,000 lily species, cultivars and hybrids that included 350 specimens established by Latvian breeders (Matthews, 2007). Because many of the cultivars that originated in Latvia are rather endangered and in limited supply; a lot of work is planned on maintenance and utilization of these plants.

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## Interspecific sunflower progenies selection for toleranceto white rot caused by Sclerotinia sclerotiorum (Lib.) de Bary

poster p4-05

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White rot caused by Sclerotinia sclerotiorum (Lib.) de Bary is a major sunflower disease worldwide. Testing *Sclerotinia* head resistance can be done using artificial infections by spraying heads at R5.1 stage with ascospore suspension using 5000 spores/ml (Becelaere and Miller, 2004). Total of 20 interspecific sunflower progenies were tested and compared with tolerant and susceptible control lines. All tested interspecific progenies were obtained after crosses between cultivated inbred lines and wild annual species, followed by one generation of open pollination due to difficulties in obtaining progenies from controlled backcross pollination. Inflorescences were screened for symptoms of head rot 35 days after inoculation. Disease severity was determined by measuring total infected area of the head. A 1-5 scale was used to describe tolerance where 1 is the most tolerant.

At the end of trial, 8 out of 9 tested Helianthus annuus progenies were highly susceptible, where symptoms of white rot covered more than 50% of the head area. Most of the H. petiolaris progenies were moderately susceptible, while the most tolerant were two progenies of H. debilis originating from accession DEB1810 where less than 12,5% of head rot was found. This study shows the possibility to use open pollinated progenies in sunflower interspecific programs where other hybridization methods are not successful. Careful selection in the following generations of backcrossing and selfing can than lead to the desired resistance trait.

Key words: ascospore suspension, head resistance, Sclerotinia sclerotiorum, sunflower

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## Natural interspecific hybrids in the genus *Lactuca* fished in nature and germplasm collections

poster p4-06

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Autogamy is the predominating breeding system within the genus Lactuca, especially in the marginal parts of distribution area (Feráková 1977). The hybridization can occur not only within one species, but also between species (Zohary 1991). Phenotypes of putative natural interspecific hybrids were recorded on primarily self- pollinated Lactuca species acquired during our collecting missions in natural habitats: L. serriola ( $\times$  L. sativa) from natural populations of L. serriola in northern Moravia; L. aculeata (× L. serriola) from Israel, and hybrid character of plants was confirmed by protein markers (Lebeda et al. 2012); L. saligna (× L. serriola) from Jordan; L. serriola (× L. saligna) from Israel. The occurrence of natural interspecific hybrids of the genus *Lactuca* in the Near East support the scientific theory that the *Lactuca* species subsection Lactuca are more allogamous near their centre of origin (Lindqvist, 1960). Their phenotype and fertility bring new theoretic information on the evolution of wild Lactuca and on the cultivated lettuce. Phenotypes of interspecific hybrids were recorded in 10 germplasm accessions within the set of 95 Lactuca spp. accessions, provided by six world gene banks. Species L. sativa, L. serriola, L. saligna, L. dregeana and L. virosa very probably participated in interspecific hybridization in combinations of L. dregeana  $\times L$ . serviola, L. saligna  $\times$  L. serriola, L. sativa  $\times$  L. serriola, L. serriola f. integrifolia  $\times$  L. sativa, L. serriola f. serriola  $\times L$ . sativa, L. sativa  $\times L$ . virosa  $\times L$ . serriola, L. dregeana  $\times L$ . sativa  $\times L$ . serriola. The most significant morphological traits of hybrids were manifested by the shape, vertical undulation and blistering of rosette- and cauline leaves, distribution pattern of anthocyanine, by the trichomes on the stems, and by the position of involucral bracts (Lebeda et al. 2007). Standards for regeneration of accessions in the gene banks should be followed. Technical isolation is highly recommended also for primarily self-pollinated Lactuca species. The taxonomic status of germplasm material provided by the gene banks should be verified before its exploitation in scientific experiments.

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## Towards Orobanche resistance in sunflower - Screening of wild Helianthus for novel resistance sources

poster p4-07

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Broomrape (Orobanche cumana Wallr.) is the most serious constrain for sunflower (Helianthus annuus L.) production in Southern and Eastern Europe. In these areas it causes considerable yield losses and reduces sunflower seed quality. Genetic resistance had proved to be the most efficient method for suppressing severe broomrape attacks in the field, however selection pressure resulted in occurrence of new, and more aggressive races of the parasite. Broomrape is absent in the centre of origin of the genus Helianthus and in the sunflower crop areas of Latin America. In Argentina, one of the five main producer countries, the parasite is also absent in the exotic populations of *H. annuus* and H. petiolaris naturally developed in the sunflower crop area (Cantamutto et al. 2010). Both natural and broad genetic resistance in Argentina sunflowers could be potentially responsible for broomrape absence in Argentina (Miladinovic et al. 2012). In order to check this hypothesis and find potential novel resistance sources we have screened wild H. annuus (N=4) and H. petiolaris accessions (N=4) from Argentina for their resistance to broomrape, as described by Terzic et al. (2010). The ANO-VA showed high significant differences between species. The tested accessions of *H. annuus* were susceptible and had  $4.3 \pm 3.3$  broomrape tassels per plant. All the accessions of *H. petiolaris* tested were resistant to broomrape as no broomrape tassels were observed. Similar results were obtained by Terzic et al. (2010) who tested *H. petiolaris* accessions from the USA. This wild *Helianthus* species could be a valuable source of broomrape resistance genes that could be incorporated into cultivated sunflower by interspecific crossing.

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### Acknowledgement

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## Tetraploid wheat wild relatives as tools of wheat improvement

poster p4-08

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Present study is focusing on the utilization of two tetraploid wheat wild relatives: *Triticum turgidum* L. ssp. *dicoccum* (Shrank ex Schübler) Thell. (emmer wheat) (2n=4x=28, A<sup>u</sup>A<sup>u</sup>BB) and *Triticum timopheevii* Zhuk. (2n=4x=28, A<sup>i</sup>A<sup>i</sup>GG). The accessions of these species preserved in the Martonvásár Cereal Gene Bank are promising sources of wheat improvement through the utilization of their characteristics and wide resistance to several biotic and abiotic stresses.

Exploitation of emmer wheat gene bank accessions has started more than 10 years ago in Martonvásár and resulted in a variety ('Mv Hegyes') which can be used not only as a source of synthetic amphiploid development, but also as a promising crop for alternative wheat growers. In recent years 56 accessions of *T. timopheevii* were characterized for the main phenotypic and resistance characters, and one (Acc. No.: MVGB845) was selected for further works. This accession was crossed with a semi-dwarf line of diploid cultivated einkorn (*Triticum monococcum* L. ssp. *monococcum* '1T-1', 2n=2x=14, A<sup>m</sup>A<sup>m</sup>), which was bred in Martonvásár, and has also remarkable resistance and other promising phenotypic characters. Most studies on the utilization of the genes responsible for resistance to the main fungal diseases have involved direct crosses between *T. timopheevii* and bread wheat (*Triticum aestivum* L.). However a new synthetic hexaploid wheat breeding stock (named *Triticum timococcum*, 2n=6x=A<sup>t</sup>A<sup>t</sup>GGA<sup>m</sup>A<sup>m</sup>) developed from the hybrids of MVGB845 and '1T-1' could ease the introgression of valuable genes into bread wheat at the hexaploid level (bridge-crossing).

After crossing the accession MVGB845 with '1T-1', the triploid hybrids were treated with colchicine to obtain a doubled, hexaploid genome. These fertile hexaploid progenies were crossed with bread wheat line 'Mv9kr1', which is carrying the recessive crossability allele kr1. Genomes of *T. timococcum*  $F_4$  plants and *Triticum timococcum*×'Mv9kr1' hybrid plants were examined with molecular cytogenetic methods (FISH, GISH), in order to identify the chromosomes of different genomes and to find translocations among them.

The research works leading to these results have received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under the Grant Agreement n°245058-SOLIBAM, and from two National Science and Technology Office projects (*TECH\_08-A3/2-2008-0397 - CONFU\_08* and *TECH\_08-A3/2-2008-0423 - ALKOBEER –* Hungary).

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## Screening of Wild Olive Germplasm for Verticillium Wilt Resistance

poster p4-09

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Verticillium wilt of olive, caused by the soilborne fungus Verticillium dahliae Kleb, represents currently the main phytosanitary limitation in olive orchards for both its destructive potential and control difficulties. In the framework of an integrated disease management strategy, the use of resistant cultivars represents a useful tool because of its low cost, efficiency and environmental friendliness. However, only a reduced number of cultivars currently in use have showed a considerable level of resistance to the development of symptoms. Wild olives may represent a useful source of genetic variability for some characters hardly found in cultivated material. A high diversity in wild populations have been recently reported from the analysis of morphological, agronomical and molecular data obtained by SSR markers, which seems to indicate their potential use for olive breeding (Belaj et al., 2007; 2011). In this work, 44 wild olive genotypes from different origins were screened in two experiments in growth chamber under controlled environmental conditions to test their resistance to Verticillium wilt. Inoculation by dipping roots in a conidial suspension of a higly virulent defoliating isolate of V. dahliae was used, including cultivars 'Picual' and 'Frantoio' as susceptible and resistant controls cultivars respectively. Disease reaction was evaluated weekly by using a 0-4 severity scale. Two (4.6%) of 44 genotypes were slightly more resistant than 'Frantio' on the basis of different disease parameters and percentage of dead plants at the end of the experiments. These genotypes will be propagated for future trials to confirm their disease reaction under controlled and field conditions. The genotypes showing higher resistance in this work could be tested in future works as rootstocks for susceptible olive cultivars of agronomic interest. Besides, they could be used as new sources of resistance in breeding programmes for resistance to Verticillium wilt. Although there is little information about the use of wild olives as genitors in breeding programs, recent studies have shown that they could additionally transmit short juvenile period to their descendants, which is a very useful character for breeding (Klepo et al., 2013).

Keywords: breeding, Olea europaea, rootstock, Verticillium dahliae

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## Genetic resources to exploit for resistance to aphids in wheat

poster p4-10

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Wheat (*Triticum aestivum* L.) is one of the most commonly cultivated crops worldwide. Three genomes compose its genetic frame (A, B and D). This allopolyploid nature (2n=6x=42) allows it to buffer for several introduced changes in its genome. Such changes have been widely exploited for the introgression of desirable characteristics into elite germplasm, either as alien translocations from various other plant species, for instance *Secale cereale* L., *Aegilops speltoides* Tausch, *Thinopyrum intermedium* (Host), *Thinopyrum ponticum* (Podp.), or as synthetic hexaploid wheats (SHW) (Friebe *et al.*, 1996). SHW are derived from the cross between the species *Triticum turgidum* L., carrying the A and B genomes, with *Aegilops tauschii* Coss., carrying the D genome.

The vast genetic diversity found among the wheat relatives is a potential source of resistance to aphids. These insects severely reduce wheat production by causing yield losses of up to 30-40% due to their direct feeding damage made to the plants. Moreover, since they are vectors of viruses, yield reductions can be up to 70% when feeding damage is combined with virus infection. Four of the most common aphid species attacking wheat worldwide are: the greenbug (*Schizaphis graminum* [Rondani]), the Russian wheat aphid (*Diuraphis noxia* [Mordvilko]), the bird cherry-oat aphid (*Rhopalosiphum padi* L.) and the English grain aphid (*Sitobion avenae* [Fabricius]) (Blackman & Eastop, 2007). Host plant resistance is the most environmentally friendly and economically sound strategy to control aphids. To date, 14 genes for greenbug resistance are reported in wheat, most of them originating from *Ae. taushii*. Eleven genes have been found, mainly in common wheat, causing resistance to the Russian wheat aphid. Only one gene has been mapped for resistance to the English grain aphid in durum wheat, whereas no resistance genes have been designated for resistance to the bird cherry-oat aphid in wheat, although there are several reports where resistance sources have been found.

Pre-breeding efforts are needed to incorporate resistance to aphids from wheat wild relatives into elite germplasm. Efficient and cost effective breeding strategies are also required, since phenotyping is one of the bottle necks in wheat breeding for resistance to aphids. A strategy to incorporate selection for aphid resistance in wheat breeding is presented.

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## Towards the Finnish LR and CWR conservation strategies

poster p4-11

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The crop wild relatives (CWR) and landraces (LR) harbour important genetic resources, which are necessary for the genetic diversity within agriculture and also for the food security of future generations. The conservation and study of the genetic diversity of CWR and LR is therefore of utmost importance.

The National Programme for Plant Genetic Resources in Finland was established in 2003 to meet the goals of the international agreements, such as Convention on Biological Diversity (CBD) and International Treaty on Plant Genetic Resources (ITPGRFA). The programme covers the genetic resources in agriculture, forestry and horticulture and aims to secure the valuable plant heritage in Finland, such as crop wild relatives, landraces, old cultivars and other valuable breeding material for future generations. However, there have not been thorough *in situ* and *ex situ* inventories of the CWR or LR within Finland. The inventories of the landraces have previously concentrated on single varieties/species or have mostly been conducted in local scale.

Our aim here is to present the integrated process towards national LR and CWR conservation strategies in Finland undertaken during the EC FPVII funded PGR Secure project. We will give short explanations of both CWR and LR inventories, priritization and *in situ* and *ex situ* gap analysis. We will aim to describe methods how the genetic resources of CWR and LR may be conserved for future use and give recommendation for the implementation of these strategies nationally. The fact that these analysis and preparation of the national conservation strategies are undertaken simultaniously will enable us to compare the similarities and differences between the processes towards the LR and CWR conservation strategies.

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## Availability of wild Brassica (n=9) in genebanks

poster p4-12

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The *Brassica* vegetables (cole crops) are a dietary staple in many parts of the world. FAO estimates that world commercial production of cauliflowers, broccoli, cabbages and other brassicas in 2011 was nearly 89 million tonnes from some 3.6 million hectares.

Wild relatives of *B. oleracea*, sharing the same chromosome number (n=9), are native to the western Atlantic and Mediterranean seaboard cliffs of Europe. They carry genes of potential value for the cole crops, such as salt resistance, insect resistance, fruit indehiscence, anti-oxidant compounds, etc. As a recent example a nutritionally enhanced, high glucosinolate content broccoli variety was launched in the UK market in 2011, derived from a cross with a wild Sicilian brassica collected in 1984 (BBSRC 2011).

Conscious of the importance of this group of species, in 2010 the ECPGR *Brassica* Working Group started an investigation of the availability of wild (n=9) *Brassica* accessions in genebanks (Maggioni and Lipman 2010). Several collecting missions had been sponsored by IBPGR between 1982 and 1988 and national or international expeditions have continued in the subsequent 25 years to survey and collect the wild populations. A search of the existing online databases, combined with additional information, indicates that only a fraction of the collected material can be traced to an *ex situ* genebank and thus requested for research and breeding purposes. Moreover, the actual availability of the *ex situ* accessions is limited or not well known. Another limitation of the existing collections is that seeds have often been collected from very few plants. Few accessions are subject to safety back up procedures. Gaps in the coverage of the entire taxonomy and geographic range of these wild brassicas have also been identified.

Improvements of the coverage and quality of the collections and an increased standardization of the procedures to ensure access for breeding and research are recommended as the result of a European coordinated effort.

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## Constraints in the Utilization of Plant Genetic Resources in the Nordic Countries

poster p4-13

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The use of plant genetic resources (PGR) held in gene banks, by breeding companies, and by research organizations is far from optimal. Therefore, as part of the EU project PGR Secure (<u>http://www.pgrsecure.org</u>), a study was initiated to identify and analyse the constraints of PGR use in Europe. As part of this study, Nordic PGR stakeholders were interviewed in 2011 and 2012. In total 14 interviews were conducted, one with a representative from the gene bank, six from public research, six from breeding companies and one from an agro-NGO.

Several strengths were identified, for example the uniquely adapted genetic resources that exists in the Nordic region. Of a specific interest are the plants' adaptations to cold temperature and day length variation at northern latitudes. Furthermore, the strong history of cooperation among the Nordic countries concerning PGR issues was highlighted as a strength. Examples of cooperation include the regional gene bank, the Nordic private-public-partnership projects in pre-breeding, and the cooperative efforts among the stakeholders to characterize and evaluate gene bank accessions.

Several weaknesses were also identified. One is the lack of easily available information on relevant traits in gene bank material, which makes it difficult for breeders and researchers to select suitable accessions. Furthermore, there is a lack of political priority for PGR questions both on the national, Nordic and European level, leading to limited opportunities to attract funding for research and development projects, including work on *in-situ* conservation. One of the future threats that was highlighted in the study is climate change, which will lead to an increased demand for varieties that are adapted to the new conditions. The genetic resources represented by landraces and crop wild relatives (CWR) are of fundamental importance to meet these future challenges. However, the breeders work on an increasingly competitive market, that in the Nordic region has resulted in a concentration on a few major crops and avoidance of the expensive and time consuming use of CWR and landraces. A joint Nordic perspective could be part of the solution, but also a better European cooperation. However, a lot of actions need to be taken on several levels in the society.

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## About introduction and naturalization of species genus *ALLIUM* L.

poster p4-14

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New direction in the conservation of biodiversity can be enriching flora zones with poor vegetation cover by introducing plant species, in the area previously not grown, but had abilities to easy adaptation to specific environmental conditions, without the threat of becoming ruderats. In this regard are promising ornamental Allium L. species of section Molium Don., geokserofite ephemeroides: A. stipitatum Regel, A. suvorovii Regel, A. aflatunense B. Fedtsh., A. giganteum Regel, A. altissimum Regel etc. They have the short period of the annual vegetation — from early spring until early July. With the same purpose are useful A. sphaerocephalon L., A. obliquum L., A. caeruleum Pall., A. caesium Schrenk etc. They practically do not require special care. The backlog of nutrients into the bulbs and rhizomes enabled their adaptation to the new conditions. Rhizomatous species of onion: A. schoenoprasum L., A. angulosum L., A. ledebourianum Roem, et Schult, and some others use for long year garden design. They are also sown as greens and cover crops. The plants of these species are extremely unpretentious, cold tolerance and winter hardiness. Because of intense branching they formed dense clumps and does not suffer from the weeds. In 1996 in Moscow were laid experiments to introduce A. altissimum Regel, over the years adapted to local conditions, natural plant communities in areas with depleted natural vegetation. The introduction was successful. One plot extended until 2011. Number of flowering plants in the season 2009 was 69, the total number of plants 108 instead 40, at first. In the year 2011 the number of flowering plants was 82. Monitoring of populations showed almost complete survival of plants and their competitive enough. In setting up similar experiments we should considered the attention of species to environmental factors; analyze microclimate, micro relief, soil conditions, composition of the existing plant community. Requires regular monitoring and reckon of population number in a few years (the age structure of the population, flowering plants, seed production, vegetative propagation coefficient, etc.) and determine the competitiveness of plant species, including seedlings in plant associations. For efficient introduction and naturalization of Allium L. species is necessary to study their seed and vegetative propagation in the zone where you plan to grow, as well as the development of recommendations for cropping seeds and sets for planting.

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# The use of *Vasconcellea* wild species in the papaya breeding (*Carica papaya* L. - Caricaceae): a cytological evaluation.

poster p4-15

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Papaya is a fruit species grown in tropical and subtropical regions. The cultivars of this species are all susceptible to ring spot mosaic virus; however, there is in the Caricaceae family wild species that exhibit resistance genes to the virus as *Vasconcellea cauliflora*, *V. cundinamarcensis*, V. quercifolia, and V. stipulata. But, interspecific/intergeneric hybrids are not obtained between the wild species and the cultivated one, thus preventing the introgression of important genes in the cultivated form. The objectives of this study were to generate cytogenetic knowledge by karyotype determination and species meiotic behavior. The study was conducted on C. papaya, V. quercifolia, V. cundinamarcensis, V. cauliflora, V. goudotiana, and V. monoecious by routine laboratory methodology. All species analyzed have eighteen chromosomes and nine pairs of bivalent chromosomes confirming the number described for Caricaceae. The chromosomes size of C. papaya ranged from 2.29 µm to 1.52 µm, V. quercifolia from 2.17 µm to 1.47 µm, V cundinamarcensis of 2.45 μm to 1.66 μm, V. cauliflora from 2.73 μm to 1.57 μm, V goudotina of 3.06 μm to 3.2 μm, and V. monoica from 2.49 µm to 1.35 µm. All species showed similar and symmetrical karyotype with nine pairs of metacentric chromosomes, except V. goudotiana that has 5 pairs of metacentric and 4 submetacentric chromosomes. Meiosis was also evaluated and was observed nine pairs of bivalents confirming that the species are diploid. Meiosis was normal, but, some abnormalities were observed, as laggard chromosomes, sticky chromosomes, precocious segregation, and lack of synchrony. The estimated recombination index ranged from 26.2 for C. papaya and V. goudotiana to 25.8 for V. monoica. The meiotic index estimated based on numbers of post-meiotic products ranged from 94.8% (C. papaya) to 77.6% (V. monoica). These indexes reflected in pollen viability which ranged from 96.0% (C. papaya) to 68.0% (V. goudotiana). Based on the results, that the karyotypes are similar and symmetrical and that the species are genetically stables, there are no differences in the chromosomal homology between species that explain the unsuccessful results registered for interspecific/intergeneric hybridization between Caricaceae species.

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## Crop wild relatives as a source of the biotic stress resistance in cultivated pea (*Pisum sativum* L.)

poster p4-16

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The genus pea (*Pisum* L.) originated in Near Eastern and Mediterranean centres of diversity. Majority of modern taxonomic classifications recognise three pea species, namely common pea (P. sativum L.), red-vellow pea (P. fulvum Sm.) and Ethiopian pea (P. abyssinicum A. Braun). Red-yellow pea belongs to the indigenous floras of Armenia, Cyprus, Greece, Iraq, Israel, Jordan, Lebanon, Syria and Turkey. It has remained completely wild species, with typical traits such as dehiscent pods, small seeds and thick testa. On the other hand, Ethiopian pea may be found solely in Ethiopia and Yemen, has a narrow genetic variation and is at least partially domesticated crop. Red-vellow and Ethiopian peas are considered examples of wild relatives that may be gene pools of desirable traits for introducing into the crops while retaining a high agronomic performance of the latter. Red-yellow pea is a source of the genes controlling resistance to pea weevil (Bruchus pisorum L.), the most economically harmful pest of pea in arid climates such as Australia or Southeast Europe (1). Both red-vellow and Ethiopian peas are also regarded as the sources of novel genes, such as er3 (2), controlling resistance to powdery mildew (Erysiphe pisi DC), as well as to Mycosphaerella pinodes. On the other hand, there are certain chromosomal differences and partial fertility between red-yellow and Ethiopian peas on one side and common pea on the other side. Such complex restrictive behaviour frequently limits a degree of the success of introgressing desirable traits related to enhancing biotic stress resistance from wild into cultivated germplasm. This is also a long process, since numerous back-crosses between an red-yellow or Ethiopian and common pea F. hybrid and common pea cultivar are needed to introgress a trait improving biotic stress tolerance and merge it with high, quality and stable grain yield. Recently, first attempts have been made in Novi Sad to introgress the resistance to pea weevil from red-yellow into common pea by crossings in field and glasshouse conditions, aiming at contributing to the knowledge on pea crop wild relatives may have in developing advanced cultivars resistant to prevailing pests and diseases in local environment (3).

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## Wild relatives of cool season food legumes in Turkey

poster p4-17

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Wild relatives of cool season food legumesare very importantas a part of genetic diversity, and provide lots of breeding benefits such as resistance to biotic and abiotic stresses. In this review, wild species of cool season food legumes including Cicer, Lens, Lathyrus, Lupinus, Pisum, Vicia, Vavilovia, and their ecogeography and distribution in Turkey were presented. The genus Cicer L. includes of C. arietinum L. (the cultivated species), C. bijugum K.H. Rech., C. echinospermum P.H. Davis, C. judaicum Boiss., C. pinnatifidum Jaub. & Sp., C. reticulatum Ladiz. (wild progenitor) as annual wild relatives and C. anatolicum Alef., C. floribundum Fenzl, C. heterophyllum Contandr et al., C. insicum (Willd.) K. Maly, C. isauricum P.H. Davis, C. montbretti Jaub. & Sp., and C. uluderensis Donmez as perennial species are distributed in Turkey's flora. The genus Lens Miller contains L. culinaris ssp. culinaris Medik. (the cultivated species), L. culinaris ssp. orientalis (Boiss.) Handel-Mazetti (wild progenitor), L. culinaris ssp. tomentosus Ladiz.,L. culinaris ssp. odemensis Ladiz., L. ervoides (Brign.) Grande, L. lamottei Czefr. and L. nigricans (Bieb.) Godron. All taxa are grown in Anatolian region. The genus Lathyrus L. is one of the largest genera and included 64 species distributed in Turkey (Guner 2012). The genus Lupinus L. includes of L. albus L., L. anatolicus W.Swiecicki & W.K.Swiecicki, L. angustifolius L., L. hispanicus Boiss. and Reut., L. micranthus Guss. and L. pilosus Murr. are found in Turkey. The taxa Pisum comprises distinctly different two species, P. sativum L. and P. fulvum Subth & Smith and all taxa are distributed in the flora of Turkey as well. The taxa of Vicia faba L. including V. faba ssp. paucijuga Murat., V. faba ssp. faba L. var. minor, V. faba ssp. faba L. var. equina, V. faba ssp. faba L. var. faba are grown in Turkey. Vavilovia Fed. is represented by only one species, Vavilovia formosa (Steven) Fed. and distributed in Turkey. Archeological remains, variation in cultivated species, wild endemic species and progenitors grown in Turkey suggested that Turkey is one of the most important variation centers and cradle of cool season food legumes and also origin center.

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## Wild wheat as source of resistance to leaf rust and powdery mildew

poster p4-18

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Leaf rust, caused by *Puccinia recondita* f.sp. *triticina*, and powdery mildew, caused by *Blumeria graminis* f.sp. *tritici*, are the most prevalent of all of the wheat diseases in many regions of the world including Poland. These two diseases can cause serious epidemics in Poland. The aim of presented research is pyramiding of leaf rust and powdery mildew resistance genes, like (Lr41+Pm21+Lr47), (Lr41+Pm21+Pm37), (Lr41+Lr47+Pm21+Pm37), (Lr41+Lr55+Pm37) and (Lr41+Lr55+Pm21+Pm37) in a one genotype.

As the donor of resistance to leaf rust several lines were used. The line KS90WGRC10, which carries the Lr39 (= Lr41) gene derived from the diploid wild wheat *Triticum tauschii* (syn. *Aegilops squarrosa*). The line KS04WGRC45, that carries the Lr55 gene derived from the *Elymus trachycaulus*. The line HRS Yecora Rojo, which carries the Lr47 gene derived from *Triticum speltoides*.

As the donor of resistance to powdery mildew two lines were applied. A 6VS/6AL translocation line of Yangmai5 that carries the *Pm21* gene derived from the wild cv. *Dasypyrum villosum* and line NC99BGTAG11, which carries the *Pm37* gene derived from the *Triticum tiomopheevii*.

To detect resistance genes (foreground selection) several molecular markers for Lr41, Pm21, Lr47 and Pm37 were applied. In addition, plant materials were inoculated in the greenhouse at the three-leaf stage with a natural pathogen population of *P. recondita* and *B. graminis*. The marker-assisted selection and resistance tests allowed to obtain lines carrying three or four resistance genes, (Lr41+Pm21+Lr47), (Lr41+Pm21+Pm37), (Lr41+Lr55+Pm37), (Lr41+Lr47+Pm21+Pm37), and (Lr41+Lr55+Pm21+Pm37) into a single genotype.

**Key words:** *Blumeria graminis*, gene pyramiding, *Lr41* gene, *Lr47* gene, marker-assisted selection, *Pm21* gene, *Pm37* gene, *Puccinia recondita*.

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## Identification of partial resistance to powdery mildew in synthetic hexaploid wheat

poster p4-19

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Hexaploid bread wheat originated from the spontaneous hybridization of tetraploid *T. turgidum* (2n = 4x = 28, AABB) with diploid *Aegilops tauschii* (2n = 2x = 14, DD) some 10 000 years ago. The genetic diversity of the D-genome of bread wheat is low, although a huge genetic diversity is present among wild populations of *Ae. taushcii*. A way to tap into this gene pool for breeding is the creation of synthetic hexaploid amphiploids by hybridization of elite durum wheats with diverse accessions of *Ae. tauschii* (Mujeeb-Kazi et al. 2008).

Here we report on the assessment of partial resistance to powdery mildew in a set of 448 primary synthetic wheat lines from CIMMYT. In 2005, the whole collection was screened for powdery mildew resistance at two locations in south-eastern Norway (Ås and Hamar) in unreplicated hillplot trials with the partially resistant and moderately susceptible checks 'Naxos' and 'MS273-150' planted in every 20th plot. Out of this rough screening, 66 lines were selected with powdery mildew severities less than 'MS273-150' in both field trials. This reduced set was then properly tested for powdery mildew resistance in replicated and randomized field trials under high disease pressure over three field seasons in Ås and Hamar in South-Eastern Norway (2006, 2007, 2008), Stjørdal in central Norway (2008) and Changins in Switzerland (2007). Since the local powdery mildew pathogen populations at these locations are known to differ in their virulence composition, stable levels of low disease severity across locations can be taken as an indication of possible race non-specific and durable partial resistance (Lillemo et al. 2010). This testing identified a subset of 11 lines with partial resistance across locations at the same level or better than the well-characterized sources of partial resistance 'Saar' and 'Naxos'. This is a valuable set of synthetic hexaploid wheat lines that can be used for diversifying the genetic basis of partial resistance to powdery mildew in bread wheat.

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## Collection of *Prunus mira* (wild peach) in the Himalayan region of India

poster p4-20

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*Prunus mira* is a wild relative of peach, growing extensively in cold/dry regions of north western Himalaya in India and southern Tibet, at elevations of 2000-4000 m over the sea level.

A tree at about 1000 years old was recently found in the Tibet region and at present it is widely used in China as a rootstock for peach growing. The hypothesis that *P. mira* could be a natural hybrid between peach and almond because it is crossable with both species has not been confirmed.

This self-fertile species it is considered to be cold and dry tolerant, as well as resistant to powdery mildew and to root knot nematodes. The interest of this species it is to introduce these traits in peach, almond and rootstock breeding programs.

In September 2012, within the framework of a national project aimed at enlarging the peach variability in Spanish collections, a team from CITA went to Baspa Valley, in Kinnaur Region, in the state of Himachal Pradesh in India to prospect this species.

As a result, 130 seeds from 13 different accessions of *P. mira* were collected, representing the wild variability of this species in this valley. Fruits, leaves and seeds were collected to make pictures and stones were removed from the fruits. Passport data, including GPS location, were recorded following the descriptors recommended by Bioversity International. Some ethnobotanical data could also be recorded thanks to collaboration of local people from the small villages of the valley.

The seeds will be germinated and the material will be used for genetic studies and pomological and molecular characterization.

This work has been financed by INIA, RF2009-0002-C04 project and A12 group from Gobierno de Aragon.

Keywords: Prunus mira, wild peach

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## Pre-breeding focused on late blight resistance utilizing a diverse potato germplasm

poster p4-21

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Potato late blight caused by *Phytophthora infestans* is the most destructive potato disease. Sources of resistance have been found in many wild potato species. Pre-breeding research with the aim to develop late blight resistant germplasm was established as a cooperation project between N.I. Vavilov Institute and the Swedish program for plant breeding of potato at the Swedish University of Agricultural Sciences (Alnarp) in 2008. Some examples of the results so far achieved are presented.

To combine high leaf and/or tuber resistance to late blight with satisfying consumer qualities accessions from five wild species (*S. guerreroense* (grr), *S. berthaultii* (ber), *S. ruiz-ceballosii* (rsc), *S. kurtzianum* (ktz), *S. neoantipoviczii* (nan)), two interspecific hybrids (*S. microdontum* × *S. tarijense* (mcd×trj), *S. tuberosum* × *S. polytrichon* (tbr×plt)) and three cultivated species (*Solanum tuberosum* (tbr), *S. tuberosum* group *Andigena* (adg), *S. tuberosum* group *Phureja* (phu)) were used in crosses. The resulting hybrids were evaluated under field conditions with no fungal treatments in 2010-2012.

Under severe natural infection pressure five out of eleven hybrid populations containing germplasm from grr, plt, nan, mcd and trj showed high frequencies of resistant plants. The progeny seedlings originating from a cross between the breeding line SW93-1015 (tbr) (expressing high foliar field resistance) and an accession of ber segregated for leaf and tuber resistance. They exceeded the tbr parent in tuber size and earliness. Progenies obtained from a cross between SW93-1015 and adg also expressed high levels of field resistance. Some clones from this combination were high yielding and showed good agronomic characters. Late blight resistance was also shown by a hybrid obtained in a cross between a selection from the Russian cultivar Aurora and an accession of rsc.

A majority of wild potato species is characterized by small tuber size. Hybrids obtained were improved regarding tuber weight compared to the wild parents except in the combination  $grr \times adg$ . An accession of the species grr was found to possess extreme foliar resistance but was characterized by complications regarding tuber production under long day conditions. A hybrid between this grr-accession and the cultivar Superb showed complete resistance and considerably exceeded the wild parent in tuber size.

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5 Genetic resources and traits to address climate change

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# Identification of candidate genes related to seedling vigor under cold conditions in rice (*Oryza sativa* L.)

poster p5-01

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Vigorous rice growth during the seedling stage under cold conditions is an important trait for stable seedling establishment in the direct seeding method in temperate areas and at high altitudes in tropical and sub-tropical areas. In order to identify the genes involved in rice seedling vigor under cold conditions, comprehensive gene expression analysis was performed with recombinant inbred lines (RILs) derived from a cross between cultivars with high seedling vigor and low seedling vigor. A gene for which expression correlated with seedling vigor under cold conditions was identified.

Seedling vigor under cold conditions of 108 RILs derived from a cross between a highly vigorous Italian rice cultivar 'Italica Livorno', and a Japanese rice cultivar with low seedling vigor, 'Hayamasari', was evaluated after growth at 17 °C for 9 days. Thirty-one RILs were found to be significantly more vigorous than 'Hayamasari', and 33 RILs were significantly less vigorous than 'Italica Livorno'. Forty-four RILs showed seedling vigor between those of 'Italica Livorno' and 'Hayamasari'. A comprehensive microarray survey with two highly vigorous RILs, two RILs with low vigor, 'Italica Livorno' and 'Hayamasari' showed that 7 genes were expressed at higher levels in the highly vigorous RILs and the cultivar than in those with low vigor. The expression levels of these 7 genes were analyzed by quantitative real-time PCR using TaqMan probes with 22 highly vigorous RILs and 20 RILs with low vigor. The results showed that the expression level of 1 of the 7 genes, a cytochrome P450 gene, was significantly correlated with seedling vigor under cold conditions. DNA sequence polymorphisms in the promoter and coding regions of this gene in 'Italica Livorno' and 'Hayamasari' are being analyzed. The correlation between polymorphisms in the promoter region of the gibberellin oxidase 1 gene and seedling vigor will be also shown.

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# Genetic variation and preservation of authochtonous legume landraces grown on saline soils from West part of Romania

poster p5-02

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Saline soils in the West part of Romania comprise almost 200.000 ha. We have prevolusly identified this type of soils by analyzing their composition in Cl-, Na+, Ca++, Mg++, K+, SO4--. and precise mapping (Ardelean et al., 2003; 2004). There is a wide variation of salinity resistance in food legumes (Vadez et al., 2007). In order to identify salt-resistance genotypes for breeding programes, interaction between genotype and environment need to be considered (Flowers et al., 2009). Although there are few salt tolerant species, some genotypes present an acceptable level of adaptability to moderate saline conditions. In legumes, these genotypes are represented especially by local landraces breeded over the time by local farmers from areas with saline soils. Many farmers have stopped growing these traditional ecotypes and turned to commercial varieties, which are fast growing and shape enhanced but not necessary adapted to local environment. Identification of these cultivars represents a priority in breeding programs of vegetables all over the world. We have collected legume landraces (Allium sativum, Allium ascalonicum, Allium cepa, Lycopersicon esculentum Mill and Phaseolus vulgaris) landraces from 2 agro-ecological regions known for their salinity from the West Part of Romania in order to assess their phenotypic and genetic variation and to preserve the traditional germplasm. We imposed two criteria for collection: seed saving method by local farmers and no crossing or tweaking for commercial reasons. The distance between each pair of populations ranged from 10 to 50 km. The number of individuals in each population ranged from 10-100. Differences between accessions were observed in the fruit traits. We employed two classes of neutral genetic markers based on polymerase chain reaction (PCR): random amplified polymorphic DNA (RAPD) inter-simple sequence repeat (ISSR) markers to assess the genetic diversity. Our preliminary results show a large variation among the genotypes and the need for accurate estimation of allele frequencies. The main goal of our study was to identify populations to which priority should be given for dynamic conservation of landraces and make a baseline data available for farmers and breeders in this area.

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# Studies of waterlogging tolerance in Nordic barley cultivars (Hordeum vulgare, L.) using chlorophyll fluorescence on hydroponically grown plants

poster p5-03

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Barley is very susceptible to waterlogging and is a limiting factor influencing barley production worldwide and with expected climate changes, its importance will increase. Today waterlogging is supposed to reduce yields with 20-25%, but could exceed 50% depending on stage of development. Similar to other abiotic stresses waterlogging tolerance is a complicated trait and the lack of useful efficient selection methods is an obstacle in breeding programmes. Genotypic variation for tolerance to waterlogging has been observed and methods used are mainly indices based on leaf chlorosis and germination ability. Most studies are done with waterlogged soils, although the use of hydroponics could be an interesting alternative. Cultivation in O2 depleted nutrient solution is the ultimate waterlogging system that can be carefully controlled contrarily to if soil is used. The objective of this study was therefore to develop a method based on hydroponically grown material. As a measure of effects of waterlogging or in this case low O2 concentration, leaf fluorescence was used. Leaf fluorescence is a very fast way to observe stress effects on the photosynthetic pathways.

A reference material of 12 cultivars with a difference of 40% in waterlogging tolerance based on studies in water logged soil was used for developing the screening method and a reasonably good correlation (r=0.85, df=10) was obtained between plant growth reduction in water logged soil and leaf fluorescence of plants grown in un-aerated nutrient solution. The method was used in a screening of 172 Nordic old and new barley cultivars. In Sweden and Denmark, the release of new cultivars until 1950<sup>th</sup> has resulted in less waterlogging tolerant cultivars. However, during the last 30 yrs this trend has changed and many new cultivars are now more waterlogging tolerant. Similar trends are also observed in Norwegian and Finish cultivars. These changes are probably an effect of an unintentionally adaptation of new cultivars to higher precipitation in late spring and early summer by breeding for yield.

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# Genome-wide analysis and expression profiling of half-size ABC protein subgroup G in rice in response to abiotic stress and phytohormone treatments

poster p5-04

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The roles of the proteins encoded by half-size adenosine triphosphate-binding cassette transporter subgroup G (ABCG) genes in abiotic stress responses are starting to be established in the dicot model Arabidopsis thaliana. In the monocot model rice, the functions of most half-size ABCG proteins in abiotic stress responses are unknown. Rcn1/OsABCG5 is an essential transporter for growth and development under abiotic stress, but its molecular function remains largely unclear. Here, we present a comprehensive overview of all 30 half-size ABCG genes in rice, including their gene structures, phylogeny, chromosome locations and conserved motifs. Phylogenetic analysis revealed that the half-size OsABCG proteins were divided to four classes. All seven rice intronless genes, including Rcn1/OsABCG5, were in Class III, like the 12 intronless ABCG genes of Arabidopsis. The EST and FL-cDNA databases provided expression information for 25 OsABCG genes. Semi-quantitative and quantitative RT-PCR analyses demonstrated that seven OsABCG genes were up-regulated in seedlings, shoots or roots following treatments with abiotic stresses (6°C, 17°C, 42°C, NaCl, or mannitol) and abscisic acid. Another 15 OsABCG genes were up-regulated under at least one of the abiotic stress conditions and other phytohormones besides abscisic acid. Hierarchical clustering analysis of gene expression profiles showed that expression of the OsABCG genes could be classified into four clusters. The Rcn1/OsABCG5 cluster was up-regulated by abscisic acid and included OsABCG2, 3, 13 and 27. The present study will provide a useful reference for further functional analysis of the ABCGs in monocots.

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# Histological Characteristics of Sugar Beet Petiole through an Evaluation of Drought Tolerance

poster p5-05

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Generally, the structural characteristics of the plant that are associated with its ability to survive under dry conditions are referred to as xeromorphic characteristics. Assessment of the variability degree of morphological, micromorphological and anatomical characteristics of breeding material with respect to water use efficiency and drought can be used for selection of sugar beet genotypes with better tolerance to water deficiency. In order to provide it, there is a need to assess the degree of genetic variability of various plant part characteristics with respect to water management in plants, under optimal water supply. The scanning electron microscopic and classical anatomical methods were used in our previous work, to complete the comparative micromorphological and histological analysis of lamina and petiole sugar beet genotype. By applying the stereological method, in this research we performed an anatomical analysis of petiole on the same 10 sugar beet genotypes as they were analyzed in our previous work by the classical anatomical procedure. The general variability of the sample, and the contribution of individual character on variability were also analyzed, as well as . A genotype specificity and significance of differences of volume densities (Vv) of various tissues were obtained for each petiole segment. A significant petiole cross-section shape variability was found in both the proximal and distal segments. Significant genotypic differences in volume density of epidermis, vascular tissue, collenchyma, sclerenchyma and parenchyma were recorded. Apart from the ratio of collenchyma, it is also important to consider its distribution, which is in relationship to a petiole shape. A correlation analysis between the petiole length and tissue volume densities showed that longer petiole had smaller values of Vv of epidermal and vascular tissues and higher values of Vv of parenchima. The results of PCA analysis showed that the percentage of parenchyma surface area and petiole index were characteristics that mostly contributed to the overall variability.

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# Studies on biodiversity of local onion (Allium cepa L.) landraces cultivated in saline areas from western Romania through phenotypic and molecular analyzes

poster p5-06

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The transformation of soils into saline ones is a frequent phenomenon in Romania and also worldwide, due to the accumulation of highly soluble salts at the surface or in the soil profile. The area covered by saline soils in Romania is 609,6 thousand ha, which represent 6,5 % of arable area.

The problems determined by salinity are associated mostly with other abiotic stress factors, as drought and phosphorus deficit. Quality improvement of these soils by using amendments associated with irrigation and drainage needs much too high investments for semi-subsistence agriculture, specific to Romanian rural areas. Other viable solutions, accessible to farmers are valuable local germplasm identification and breeding, which can be realized due to technological evolution and improvement of modern biochemical, physiological and molecular analytical instruments. Thus, the understanding and using of the genetic basis of cultivated species tolerant to salinity is possible.

Around 80 onion landraces adapted for saline soil conditions were collected and analyzed, from an area covering around 150.000 ha from Western part of Romania. Phenotypic characterization were based on biometrical measurements on onion bulbs traits.

As a tool for genotype characterization and assessment of genetic diversity, DNA fingerprinting was used for all of the landraces.

ISSR (Inter Simple Sequence Repeat) markers were used, because it is a simple and quick method that combines most of the advantages of microsatellites (SSRs) and amplified fragment length polymorphism (AFLP) to the universality of random amplified polymorphic

DNA (RAPD). ISSR markers are highly polymorphic and are useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology.

The genetic similarity coefficients and dendogram, reflecting genetic relatedness of the landraces were established based on Inter simple sequence repeat (ISSR)-PCR fingerprint. Cluster analysis based on Jaccard's similarity coefficient using UPGMA grouped the landraces into different clusters, which were correlated with the phenotypic data.

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# Adaptation to cultivation in a Swedish climate - a study of landrace field pea

poster p5-07

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Pea is among the historically most cultivated species in Sweden and field peas were for a long time of major importance for human consumption. Although plant improvement of field pea began in the late 19th century, landrace peas were still of major importance well into the 20th century, possibly due to them being well-adapted to the local growing conditions. We have studied Swedish field peas from three different sources: Peas preserved *ex situ* in genebanks, peas preserved *in situ* on farm in active cultivation, and non-actively maintained peas stored as harvest seed samples for more than 100 years.

A screen of neutral genetic markers showed a substantial loss of genetic diversity during the past 100 years. It also showed how the abandonment of traditional farming methods, leading to more fragmented populations, has affected the Swedish pea gene pool. Analysis of genes controlling flowering time revealed substantial local adaptation in preserved landraces and major breeding resources for cultivation of pea beyond the present range of modern pea farming.

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# Advanced approaches to the analysis of genes and alleles influencing growth, development, and yield components of wheat, with the aim of defining better adapted genotypes

poster p5-08

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Recombinant inbred line mapping populations of wheat have been produced with the aim of searching for better adapted combinations of genes and alleles influencing yield. These mainly include genes and alleles controlling growth habit and flowering time, and resistances to biotic and abiotic stresses.

Two mapping populations based on substitutions of chromosome 3B of the Czech alternative landrace, Ceska Presivka, carrying a novel flowering time gene, QFt.cri-3B, have been subjected to detailed genetic and phenotypic analyses of the 3B chromosome region of interest. This region spans a large genetic distance between the markers Xgwm285 and Xcfa 2170, and fine mapping using segmental recombinants is being carried out to genetically dissect the region.

A further three mapping populations have been produced based on crosses between three different genotypes of wheat, representing different genotype pools grown under the climatic conditions of the Czech republic,. These are being studied to evaluate the existing allelic variation, so as to discover the most favourable combinations of genes and alleles, for optimal growth and yield in the country.

Acknowledgement:

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# Analysis of Experimental Design for Phenotyping by Drought and Heat Resistance

poster p5-09

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Genetic resources have historically been characterized by taking data in the field, but recently, genotyping has become cheaper and easier than phenotyping. This change has necessitated linkage of genotypic and phenotypic experimental design and analysis strategies, with designs focusing on evaluation of large numbers of genotypes. The Seeds of Discovery Initiative has as one of its priorities the identification of germplasm and alleles to address climate change. In this paper we present the strategy we followed for phenotyping characterization of the testcrosses of the CIMMYT maize Genebank Breeder's Core Collection for heat and drought tolerance. The experimental design used was an augmented row column design that included four checks replicated across the experiment in a balanced way in rows and columns. Two of the checks were commercial hybrids, the other two were tolerant and one susceptible (to heat or drought). Accession/testcrosses were not replicated because the main interest was to sample haplotypes or SNPs. Paired experiments, irrigated and water stressed, were conducted on the same testcrosses. Two steps and one step analysis were compared to analyze the data and to produce new traits related to heat and drought tolerance. First, we analyzed each trial separately to obtain BLUPs for each accession and the corresponding matrix of variance covariance. Results for each trial are combined using the variance-covariance matrix as weight to produce new traits. In the second approach the data of the two paired experiments is combined in a single analysis and the matrix of correlation between traits and trial is modeled. In both approaches, the spatial variability was modeled by an autoregressive first order model in rows and columns. A number of paired trials were realized in different environments (year or site), then a combined analysis across environments also was performed. From a statistically point of view, combined analysis is more efficient because it incorporates all information and the correlation between environments and traits can be modeled. In the two step analysis some information is lost as opposed to the one step analysis in which information from several sources is combined and improves predictions.

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# Yield-forming traits connected with drought resistance in barley

poster p5-10

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POLAPGEN-BD (www.polapgen.pl) is co-financed by EU and is the largest coordinated plant genomics project in Poland. Its subject is drought resistance in cereals, investigated in spring barley as a model plant. Spring barley is more vulnerable to drought than the winter genotypes and the most significant in Polish agriculture among spring cereals. The project stems from increasing desiccation of the environment observed mainly in the Polish Lowland. Breeders do not have tools that would enable selection of plants resistant to drought. Development of such tools could accelerate biological progress. The study will provide knowledge on the variation of morphological, anatomical, physical and physiological features of spring barley grown in controlled conditions and subjected to drought stress, in conjunction with the responses at molecular level. The project consists of 23 tasks, carried out by ten research units and two breeding companies, covering a wide range of characteristics determining plants resistance to drought. A systems approach is achieved by adopting a model of tolerance to drought stress containing ecophysiological, morphological, anatomical, metabolic, proteomic, and molecular levels considered in the context of genetics. The results of the studies will concern localization of loci linked with ability to good yielding under shortage of water, the identification of genes associated with resistance, gene expression in drought, physiological parameters related with adaptation to water shortage, and quantitative and qualitative changes in proteins and metabolites under stress. Data obtained will allow creation of ideotype of resistant barley variety. Molecular markers and methods developed as the results of this project will be applied by breeders for selection of genotypes resistant to water shortage.

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# Past and present population genetic processes in local populations of *Phleum pratense* L. – phylogeography, genetic structuring and local adaptation

poster p5-11

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The predicted climate changes poses a challenge for all living organisms and the potential for rapid adaptation to new climatic conditions will be crucial for the survival of many species. The basis for adaptation by natural selection is genetic diversity and variation in relevant traits. Genetic diversity in wild relatives of cultivated species can be utilized in the broadening of the genetic base for development of improved cultivars with better adaptation to future climates.

Timothy (*Phleum pratense* L.) is the most important forage grass species in Northern Scandinavia. We analyzed genetic and phenotypic diversity in local populations covering the entire geographic distribution range of timothy by analyses of SSR, cpDNA sequence and phenotypic variation of 14 morphological and phenological characters. The distribution of cpDNA variation indicates a classic post-glacial expansion pattern with most populations originating from two well-known refugia, but also a few populations having survived in a more northerly, cryptic refugium. No signs of geographic structuring of SSR-variation were evident indicating a large inherent effective population size, possibly in combination with extensive gene flow. No reduction in neutral genetic variation was found in peripheral populations compared to central European populations. In contrast to the lack of geographic structuring of variation in neutral markers, phenotypic variation was clearly structured and indicates natural selection and local adaptation. This points out the importance of utilizing several marker systems when assessing genetic diversity, specifically when it comes to evaluating adaptive potential. The results will be discussed in relation to breeding and conservation of genetic resources of timothy.

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6 Genetic endowments for reducing agriculture's footprints

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# Evaluation of morphological traits and biological characteristics of the wild Ukrainian ecotypes of perennial grasses in Lithuania

poster p6-01

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During the period 2007-2012 forage and turf grass genetic resources were collected in natural or semi-natural habitats of western and northern parts of the Ukraine (Precarpathian, Carpathian, Za-carpathian and Polese regions). A total of 324 seed accessions from 34 perennial grass and legume species in 42 natural or semi natural habitats were collected during the expeditions. Natural habitats of valuable ecotypes of forage and turf grasses were distinguished. Perennial grass species were collected and tested according to the international research methodology (Tyler, 1987; Guarino et al., 1995).

Wild ecotypes of various herbage species were assessed for intrapopulation and interpopulation polymorphism of 16 characters. Correlation coefficients estimated for the main morphological and VCU characters showed earliness, plant height, habitus of plant, susceptibility to rust and leaf spots to be the most variable characters.

The eight narrow-leaved *Festuca* species - *F. filiformis* Pourr., *F. ovina* L., *F. pallens* Host, *F. psammophila* (Hack. ex Čelak.) Fritsch., *F. pseudovina* Hack. ex Wiesb., *F. macutrensis* Zapał., *F. valesiaca* Schleich. ex Gaud. and *F. rupicola* Heuff. are rare (except for *F. ovina*) in West Ukraine's Precarpathian region. These species were found to grow only in specific habitats, i.e. on hill and small mountain slopes, on sands and calcareous mountain rock exposures. According to such morphological traits as shortness, leaf narrowness and colour they belong to ornamental, turf-type grasses.

Both tall, high yielding genotypes, suitable for forage and small, narrow-leaved and low yielding genotypes suitable for turf grass breeding were identified in such species as *Lolium perenne* L., *Poa pratensis* L. *Festuca rubra* L. etc.

According to a whole set of agromorphological characteristics, about 2.5% of the wild ecotypes of various species were found to be promising for the application in the forage or turf grass breeding.

#### Acknowledgements

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# Evaluation of agrobiological traits of switchgrass (*Panicum virgatum* L.) as a feasible energy crop in a cool temperate zone

poster p6-02

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This study is aimed to evaluate the collection of switchgrass (*Panicum virgatum* L.) as a renewable energy source for biofuel production by clarifying and comparing their productivity and other agrobiological traits with those of the red canary grass variety 'Chiefton'.

Searching for novel energy crops, an interest in C4 type plant switchgrass (SWG) has increased worldwide.  $C_4$  perennial grasses are being considered for bioenergy because of their high productivity and low inputs (McLaughlin and Kszos 2005). SWG as an energy plant is used for direct combustion, second generation bioethanol and biogas production (Parrish et al. 2012). Seeds for the germplasm collections of switchgrass were obtained from the Plant Genetic Resources Conservation Unit of the United States Department of Agriculture. The tests involved germplasm collection of 3 commercial varieties and 17 wild ecotypes of SWG.

The SWG collection was composed of the populations of three ploidy levels: tetraploids, octaploids and of mixed ploidy. A comparison of 18 SWG accessions cut once and twice per season showed those cut once per season at late stage of development to produce a higher biomass yield. Three accessions of tetraploid SWG produced the highest dry matter (DM) yield at both cutting regimes, namely the variety 'Summer' and 2 wild ecotypes No. 46 (PI 642295) and 60 (PI 642309). According to the DM yield per plant (one cut per season in the second year of herbage use), the variety 'Summer' and wild ecotypes No. 46 and 60 produced significantly (P<0.05) higher yield (28.4, 27.3, and 25.5 %) compared with the average SWG value of the trial (509.2 g) and significantly (P<0.01) more productive than the red canary grass variety 'Chiefton' (454.5 g). Measurements of the chlorophyll index (ChI) showed a possibility to estimate plant diversity within populations. Moderate correlations were estimated between leaf colour and leaf erectness (r=- $0.414^*$ ) and between leaf colour and chlorophyll index (r=0.550^\*). ChI at the beginning of heading strongly correlated with N content in the SWG biomass cut at this stage (r=0.853^\*\*).

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# Determination of Hybrid Vigor and General Combining Ability for Some Traits and Yield of Tomato (Solanum lycopersicum L.) Inbred Lines Suitable for Greenhouse

poster p6-03

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Determination of general combining ability of parents is very important in hybrid breeding. This study was carried out to investigate the genetic structure of the 30  $F_1$  hybrid tomato combinations established from 15 female lines and two male testers in order to determine general combining ability of parents and to evaluate the hybrid vigor. The 47 genotypes (30  $F_1$  and 17 parents) were planted in a randomized complete block design with three replications in a greenhouse at the Bati Akdeniz Agricultural Research Institute, Antalya, Turkey during the spring growing season of 2009.

General combining ability and hybrid vigor were investigated for ten traits. The best general combining ability for yield per plant, earliness, fruit weight, fruit firmness was obtained from 135-BH, 28-BH, G-8 and 28-BH, respectively. Additive gene action was significant for fruit firmness and soluble solid content, whereas non-additive genetic variance was significant for other eight characters.

Heterosis values varied from -36.59% to 54.10%, while heterobeltiosis values were found between -50.83% and 29.85% in yield per plant. Heterosis and heterobeltiosis for other traits were also determined negative and positive values.

As a result, lines 4-BH, 28-BH, 37-BH, 53-BH, 102-BH, 135-BH, G-8 and tester 2-T were recommended as potential lines for future hybrid breeding programs based on general combining ability for all traits.

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# Performance of Estonian native turfgrasses on lawns

poster p6-04

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Great exploitation potential of indigenous grass genepool is approved by the fact that five out of six registered Estonian turfgrass varieties originate from (semi)natural grasslands. The collection of grass accessions preserved at the Genebank of Jõgeva Plant Breeding Institute is continuously replenished with the samples from collecting missions since 2002.

Two turf trials were carried through in 2004–2009 and 2009–2012 with the purpose of rating the suitability of Estonian grass ecotypes from 12 species for lawns. The management featured mowing at a height of 3–4 cm at one to two weeks interval and compound fertiliser applications equivalent to N 130, P 20, and K 70 kg ha<sup>-1</sup> annually. The accessions were ranked based on their general impression – a combined value that considers leaf fineness, sward density, colour, disease resistance, uniformity of growth, and winterhardiness.

From amongst 158 native samples, we selected 10 accessions of *Festuca rubra*, 2 *Poa pratensis*, and 1 *Agrostis capillaris*, which exceeded the mean ornamental value of respective Estonian check varieties. If 5.0 points designates maximum performance, the highest mean scores across the trials were assigned to accessions of *F. rubra* ssp. *commutata* (4.2), *Deschampsia caespitosa* (4.1), *P. pratensis* (4.0), *F. ovina* (3.8), and *A. capillaris* (3.5). The scores for *D. flexuosa*, *F. arundinacea*, *P. subcaerulea*, *P. nemoralis*, *P. compressa*, *Briza media*, and *Phleum phleoides* remained between 1.1–3.3. The lawn trials showed that *F. rubra* has the highest potential to be utilised in indigenous turfgrass breeding in Estonia. This species is easily procurable from a wide range of habitats all over the country and holds sufficient phenotypic variability for selection.

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# Screening plant genotypes, based on ice nucleation activity, by infrared image analysis

poster p6-05

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Climate change poses new challenges to plant breeders and new threats for the humanity. Today, more than ever, we need fast and accurate screening assays, that would identify plants with desirable traits, primarily tolerance or resistance to biotic and/or abiotic stresses. A semi high-throughput screening platform, involving freezing of leaf discs in microplate wells prefilled with distilled sterile water, is presented. Ice nucleation is initiated at subzero temperatures and by ice nucleators of plant or microbial origin. The latter refer to epiphytic ice nucleation active (INA) bacteria and fungi. The microplate freezing platform is suitable for dissecting the role of each of these factors alone or in combination. It can serve as a reference point for measuring the ice nucleation activity of plant samples alone or with INA epiphytic microbes on them, or for testing the ice nucleation activity of microbes per se. The semi high-throughput screening platform consists of a cooling water-alcohol bath and a thermal camera mounted on the top of the bath. The platform's current capacity reaches a total of 768 plant samples, which can be screened simultaneously. Thermal analysis of recorded data is facilitated by the use of software that we have developed specifically for experiments in microplates.

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# Genetic improvement of *Lepidium campestre* using gene technology

poster p6-06

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Nutrient leaching is one of the main problems caused by modern agriculture due to the excessive use of nitrogen-based fertilizers and tillage. Planting of biennial or perennial crops could alleviate this problem, but suitable crop species for such purpose are very limited. Lepidium campestre is a potential catch and oilseed crop with a high yield potential (5 ton/ha). It is cold-hardy and has good features like upright stature, synchronous flowering, and resistance to logging. However, some important traits of this species need to be altered before L. campestre can be of economically viable. One serious problem is that it is prone to pod shatter with seed loss up to 50%. Another problem is lepidium has low seed oil content, only around 20% compared to 45% in winter rapeseed. Moreover, its seed oil contains high amounts of erucic acid that is not suitable for food consumption and high linolenic acid which is sensitive to oxidation. Successful genetic modification of these traits through genetic engineering relies on the availability of access to efficient regeneration and transformation protocols. However, there is no transformation method available for L. campestre. We have very recently established an efficient regeneration and transformation protocol for this species with close to 100% of regeneration frequency and an average 2-5% of transformation efficiency. The transgenic lines with both marker and target genes were confirmed by PCR and Southern blot analyses and the preliminary results showed that the oil composition in the seed oil could be altered.

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# Molecular Breeding of Crambe abyssinica for Ultra-high Erucic Acid

poster p6-07

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*Crambe abyssinica* is an oilseed crop that contains about 60% erucic acid (22:1), which is a major feedstock for the oleochemical industry. Development of oil crops with high 22:1 is thus of great commercial interest, and further increase in 22:1 will add value to it. Through manipulating three important genes involved in the fatty acid biosynthesis, we have developed transgenic Crambe lines with high erucic acid level (76%). The aim of this study is, by modifying another three key genes involved in seed lipid biosynthesis, to further increase the erucic acid content. Up to now, the candidate genes have been cloned from Crambe and RNAi constructs with seed specific napin promoter have been obtained. Transgenic lines with these RNAi constructs have been recovered through *Agrobacterium*-mediated transformation. The preliminary results showed that the multiple-gene-RNAi constructs could tailor the fatty acid composition in the seed oil. Further screening and related gene expression analysis are under way.

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# Notes

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#### About Lantmännen

Lantmännen is one of the largest Groups within the food, energy, and agricultural industries in the Nordic region. The Group, owned by 33,500 Swedish farmers, operates in 19 countries, has over 10 000 employees and a turnover of SEK 36 billion. Lantmännen Lantbruk is the core activity within Lantmännen. We develop and offer products and services that promote a strong and competitive agriculture industry. We offer our agricultural customers products such as feed, seed, fertilizer, plastic, nets and crop protection. Our sales to industrial customers comprise grain and oilseed. Read more on www.lantmannen.se



#### About Lantmännen SW Seed

Lantmännen SW Seed is an international plant breeding and seed company. The core business is the development and marketing of new plant varieties and the production of seed. Cereals, oil seeds and forage grasses & legumes are the main crops. The most important markets are located within Central and Northern Europe. *For more information, please visit us at www.swseed.com* 



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