The Max Plank Institute for Plant Breeding Research
invites you to the

17. Conference of the Genome Research Working Group Gesellschaft für Pflanzenzüchtung

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Gesellschaft für Pflanzenzüchtung

Crop Genomics and Plant Breeding
Max Planck Institute for Plant Breeding Research

11.2.2014 -13.2.2014

Programme

Tuesday 11.2.2014

13.00-13.20 hrs **Opening Remarks**:

Maarten Koornneef, George Coupland, **Max Planck Institute for Plant Breeding Research**

Christian Jung, **President of the GPZ**

Klaus Pillen, **Chair Genome Research Working Group, GPZ**

Opening Lecture

13.20 -14.05 hrs **Prof. Jiming Jiang, University of Wisconsin-Madison**
"Centromere evolution: A potato model"

Session: Quantitative Genomics

14.05-14.35 hrs **Rod Snowdon, Justus Liebig University Giessen**
"Multidimensional genomics of complex trait regulation: Examples in rapeseed and sorghum"

14.35-14.55 hrs **Vera Draba, Martin Luther University Halle-Wittenberg**
"The barley nested association mapping (NAM) population HEB-25: resistance against Rhynchosporium secalis"

14.55-15.15 hrs **Corinna Liller, Max Planck Institute for Plant Breeding Research**
"Mapping of a QTL for awn length using a novel barley multiparent mapping population"

15.15-15.45 hrs Coffee Break

15.45-16.15 hrs **Jochen Reif, IPK-Gatersleben**
"Predicting phenotypic performance in hybrid wheat"

16.15-16.35 hrs **Bianca Büttner, Bavarian State Research Center for Agriculture (LfL)**
"Fine mapping of the Rrs1 resistance locus against scald in barley"

16.35-17.05 hrs **Korbinian Schneeberger, Max Planck Institute for Plant Breeding Research**
"Impact of meiotic crossover and gene conversion on the genomic landscape of Arabidopsis recombinants"

17.05-17.25 hrs **Stefanie Pencs, Martin Luther University Halle-Wittenberg**
"Towards cloning thresh-1, a domestication gene causing a difficult to thresh phenotype in wild barley germplasm HEB25"

17.25-17.45 hrs **Wiebke Sannemann, University of Bonn**
"Identification of epistatic effects in a barley MAGIC population - understanding phenotypic variation in complex traits"

17.45-18.05 hrs **Bernd Weisshaar, University of Bielefeld**
"An update on the sugar beet genome"

Opening Reception and Poster Session - Finger Food

Wednesday 12.2.2014

Session: Crop Plant Development

9.00-9.30 hrs **Christian Bachem, Wageningen UR**
"The humble potato goes globetrotting: The escape from day-length regulation of potato tuberisation"

9.30-10.00 hrs **James Cockram, NIAB Cambridge**
"Genome-wide association analyses in wheat and barley"

10.00-10.30 hrs **Maria von Korff, Max Planck Institute for Plant Breeding Research**
"The circadian clock and photoperiodic flowering in barley"

10.30-10.50 hrs **Birgit Kersten, Thünen Institute of Forest Genetics**
"The sex-linked region in Populus tremuloides Turesson141 corresponds to a pericentromeric region of about two million bp on P. trichocarpa chromosome 192"

10.50-11.30 hrs Coffee break

11.30-12.30 hrs	**Keynote lecture: Gary Muehlbauer, University of Minnesota** **"Developing and exploiting genomics tools for barley improvement and gene discovery"**
12.30-13.30 hrs	Lunch break

Session: Genome Evolution of Crop Plants

13.30-14.15 hrs	**Keynote lecture: Catherine Feuillet, Bayer Crop Science** **"The wheat genome sequence: 17 Gb of opportunities for wheat improvement"**
14.15-14.45 hrs	**Karl Schmid, University of Hohenheim** "Population genomic and transcriptomic analysis of local adaptation in wild barley"
14.45-15.05 hrs	**Niels Müller, Max Planck Institute for Plant Breeding Research** "Natural variation in circadian rhythms suggests an effect of domestication on the circadian clock of tomato"
15. 05-15.35 hrs	**Andreas Weber, Heinrich Heine University Düsseldorf** "Towards engineering C4 photosynthesis: Lessons from transcriptomic and evolutionary analyses"
15.35-16.00 hrs	Coffee break

Session: Technical innovations in Plant Breeding

16.00-16.45 hrs	**Keynote lecture: Jochen Kumlehn, IPK-Gatersleben** **"Haploid and transformation technology in cereals"**
16.45-17.05 hrs	**Wolfgang Schweiger, Institute for Biotechnology in Plant Production, IFA-Tulln, BOKU** "Subgenome-specific expression analysis of fusarium-responsive genes in Triticum aestivum"
17.05-18.30 hrs	**Award of the Wricke Research Prize for Applied Research and Plant Breeding**
19.00-21.00 hrs	**Dinner**

Thursday 13.2.2014

Session: Technical innovations in Plant Breeding - continued

9.00-9.20 hrs	**Wolfgang Ecke, Georg August University Göttingen** "Identification of Intervarietal Substitution Lines of Rapeseed with Enhanced Embryogenic Potential"
9.20-9.40 hrs	**Björn Rotter, GenXPro GmbH** "Massive Analysis of cDNA Ends (MACE), a reduced complexity high-throughput transcriptome analysis method for simultaneous gene expression analysis and genomic mapping (TranSNiPtomics) in species with known and unknown genomes"
9.40-10.00 hrs	**Iris Finkemeier, Max Planck Institute for Plant Breeding Research** "Quantitative analysis of post-translational modifications in the Arabidopsis proteome"

*Session: Translation of *omics into breeding applications*

10 .00-10.30 hrs	**Keynote lecture: Christiane Gebhardt, Max Planck Institute for Plant Breeding Research** **"Discovery of diagnostic DNA markers for complex agronomic traits of potato by combining comparative proteomics/transcriptomics with association genetics"**
10.30-11.00 hrs	Coffee break
11.00-11.20 hrs	**Christian Obermeier, Justus Liebig University Giessen** "Mining QTL for candidate genes involved in resistance of oilseed rape against *Verticillium longisporum* by an integrative omics approach"
11.20-11.40 hrs	**Claude Urbany, KWS SAAT AG, Einbeck** "A walk on the *Omics* side …" – An integrative approach to investigate iron deficiency associated chlorosis in Maize"
11.40-12.00 hrs	**Tanja Gerjets, proWeizen** "The German Wheat Research and Breeding Alliance"
12.00 hrs	**Final remarks and end of the meeting**

Oral Presentations

Opening lecture:

Centromere evolution: A potato model

Jiming Jiang
Department of Horticulture, University of Wisconsin-Madison, USA

Centromeres are composed of long arrays of satellite repeats in the majority of plant species investigated to date. These repeat-based centromeres are believed to have evolved from "neocentromeres" that originally contained only single or low copy sequences, including centromeric genes. However, the evolutionary path from neocentromeres to repeat-based centromeres has been elusive. It is unclear how satellite repeats emerge and homogenize among different centromeres. Potato (*Solanum tuberosum*, 2n=4x=48) provides a great model system for studying centromere evolution because each of its 12 centromeres contains distinct DNA sequences, allowing comparative analysis of individual centromeres among related species. We have recently conducted genome-wide sequence analysis of the centromeres in *Solanum verrucosum*, a wild species closely related to potato. Four centromeres in *S. verrucosum* contained distinct satellite repeats derived from retrotransposon-related DNA sequences. Strikingly, the same four centromeres in potato contain either different satellite repeats or exclusively single and low copy sequences. These results show that centromeric DNA sequences diverge rapidly among closely related species, and centromeric satellite repeats may undergo boom-bust cycles before a favorable repeat is fixed and homogenized in the population.

Multidimensional genomics of complex trait regulation: Examples in rapeseed and sorghum

Rod Snowdon
Department of Plant Breeding, Justus Liebig University Giessen, 35392 Giessen, Germany

Many valuable agronomic traits are under complex genetic control by large numbers of interacting quantitative trait loci (QTL), which can have variable effects depending on the specific genetic background and the environment. In some cases trait expression is further complicated by multi-level environmental interactions. For example, seedling emergence and vigour traits can be strongly influenced not only by the genotype and the environment in which the seed germinates, but also by the environment in which the seeds have matured on the maternal plants. Heritability for emergence traits can therefore differ considerably over different seedlots, hence their genetics and inheritance are poorly understood and selection gains are low. We are applying multi-dimensional *omics* approaches to better understand the genetics of seedling emergence and vigour, particularly under abiotic stress constraints, in sorghum and rapeseed. Deep phenotyping is applied to correlate target traits showing high environmental variance to less complex, more heritable surrogate traits. Biparental QTL mapping is combined with genome-wide association analyses to dissect interesting variation in large, well-characterised mapping populations and genetic diversity panels. Finally, systems-biological analyses implementing sequencing-based transcriptome data and detailed metabolome data allow deeper insight into the molecular mechanisms of trait control on a gene network and regulatory level. By layering multiple levels of information about quantitative traits and their regulation onto genome sequences, we hope to ultimately refine genomic selection models in order to improve selection gains in breeding. In the amphipolyploid genome of *Brassica napus* the additional complexity of extensive genome rearrangements must also be considered

The barley nested association mapping (NAM) population HEB-25: resistance against *Rhynchosporium secalis*

Vera Draba[1], Frank Ordon[2], Doris Kopahnke[2], Günther Schweizer[3], Bianca Büttner[3], Andreas Maurer[1], Florian Schnaithmann[1], Klaus Pillen[1]

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[3]Bavarian State Research Center for Agriculture, Institute for Crop Science and Plant Breeding (LfL), 85354 Freising, Germany

Wild barley is an important resource for new resistance genes against different barley diseases. One possibility to tap into the wealth of this genetic diversity is the recently implemented nested association mapping (NAM) approach (Yu et al. 2008, Genetics 178, 539-551; McMullen et al. 2009, Science 325, 737-740).
The special design of the necessary NAM population, where one adapted recipient is crossed with many donors, enables to study the allelic diversity among the donor lines. The barley NAM population HEB-25 has been developed during the last years, consisting of 1,420 lines, originating from crosses of the spring barley cultivar Barke with 25 accessions of wild barley (*Hordeum vulgare* ssp. *spontaneum* and *H. v.* ssp. *agriocrithon*). The complete NAM population has been genotyped with the barley Infinium iSelect 9k SNP chip (Illumina), interrogating the allelic inheritance of 7,864 SNPs in total.
In cooperation with LfL Freising and JKI Quedlinburg seedlings and young plants of the HEB-25 have been phenotypically characterized in regard to resistance against the important barley diseases leaf blotch/scald (*Rhynchosporium secalis*), leaf rust (*Puccinia hordei*) and net blotch (*Pyrenophora teres*). In addition, we examined the reaction of adult plants against leaf rust and powdery mildew *(Blumeria graminis)* in two field trials. The data obtained were subjected to genome wide association studies (GWAS) in order to locate the exotic genes which confer pathogen resistance across the whole NAM population or within individual NAM families of HEB-25. In our talk we will present GWAS results for leaf blotch/scald.

Fine mapping of a QTL for awn length in barley (*Hordeum vulgare* L.)

Corinna Liller, Sieglinde Effgen, Marianne Harperscheidt, Maarten Koornneef

Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany

In barley (*Hordeum vulgare* L.) diversity is much higher in landraces than in modern cultivars, it is even higher still in wild barley populations. Hence the genetic diversity of these elite cultivars is very narrow and the range for adaptation to environmental conditions and stresses is limited. Introduction of genetic diversity from landraces and/or wild species might be vital for crop breeding in order to increase the capability to adapt to changes in environmental conditions.
Many studies indicate that in barley the spike is the main provider of photoassimilates during grain filling – and that the contribution of the awn is up to 90%. This makes awn length not only a morphological trait, but also a potential yield component. So far no genes that control awn length have been cloned from rice due to its loss of awns during domestication – and in barley, only one gene, termed *short awn 2* (*lks2*), has been cloned so far.
A mapping population was generated by crossing, backcrossing and intercrossing using barley cv. Morex and four wild barley lines (three lines ssp. *spontaneum* from Turkey, Iraq and Israel and one line ssp. *agriocrithon* from China). >900 Recombinant inbred lines (RILs) of this population (F_6) were used for QTL mapping (based on the BOPA consensus map of barley) of a large number of traits including awn length. Mapping resulted in several putative QTL positions of this trait, including one highly significant double QTL on the long arm of chromosome 7H, approximately 50 cM away from the *short awn 2* locus.
The QTL position was confirmed using heterogeneous inbred families (HIFs) and the offspring of two HIFs was used for fine mapping with recombinants in the region of interest. With these, the QTL location could be narrowed down to a ~10 cM region close to the telomere.

Predicting phenotypic performance in hybrid wheat

Jochen Christoph Reif

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), 06466 Gatersleben, Germany

One important challenge in hybrid wheat breeding is the development of accurate methods to predict hybrid performance before evaluating crosses in intensive field trials. We developed association mapping, ridge regression best linear unbiased prediction, BayesA, BayesB, BayesC, and BayesCπ approaches to predict hybrid wheat performance using genomic data. We studied the accuracy of the developed prediction approaches using a vast mapping population comprising 1.604 hybrids and their 135 parental lines. The 1.739 genotypes were evaluated in multi-location field trials for important agronomic traits such as grain yield, plant height, flowering time, biotic stress resistance, frost tolerance, as well as quality traits. Moreover, the parental lines were genotyped with a 9k and a 90k SNP array. The high cross validated accuracies clearly underlines the potential of genomics based prediction of hybrid performance in wheat.

Fine mapping of the Rrs1 resistance locus against scald in barley

Büttner B (1), Silvar C (2, 5), Casas A (2), Igartua E (2), Mayer K (3), Bolger T (4), Usedal B (4) Schweizer G (1)

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Rhynchosporium commune (formerly R. secalis), the causative agent of leaf blotch or leaf scald, is still one of the most important foliar diseases of barley. Yield losses attributed to this pathogen commonly range around 5-10%, though losses of up to 40% have been reported. Its high genetic variability and recombination frequency enable it to quickly overcome monogenic resistances, therefore diagnostic markers for pyramiding major resistance genes are needed very urgently for barley breeding programs. To date, four major scald resistance genes have been mapped in cultivated barley (Hordeum vulgare ssp. vulgare), and another four in wild barley (Hv. spontaneum) or Hv. bulbosum. The most abundant and effective one is the Rrs1 resistance locus, formerly known as Rh-Rh3Rh4 locus. It was mapped to the centromeric region of chromosome 3H. However, it is still not clear whether Rrs1 is a collection of several R-genes close to each other or several alleles of the same gene.
In case of Rrs1, scald resistance to R. commune was detected in the landrace SBCC145 and SBCC154 of the Spanish Barley Core Collection (SBCC) and to analyze the genetic basis in more detail two large DH mapping populations were developed crossing each donor line with cv. Beatrix. A QTL-analysis was conducted in both populations phenotyped for scald resistance in a well-established greenhouse test and selected sub-population in the field. One large single QTL was detected on chromosome 3H close to the centromere in both populations and, therefore, confirmed this locus as the only resistance locus in both populations. To confirm and enclose this locus, the “Rrs1 region” has been saturated with all available SSR and SNP-markers and a consensus map was constructed. New markers for this region are developed based on the llumina iSelect custom 9K barley chip, the barley genome zipper and a BSA analysis with AFLP. The genome zipper identified several candidate genes. Sequence analysis and mapping of the candidates is in progress. Because of a low gene/cM density, an enrichment of candidate sequences in the region of Rrs1 is done by a BSTA (bulked segregant transcriptome analysis) with four normalized cDNA libraries and Illumina HiSeq in combination with a high resolution mapping program. For fine mapping and haplotyping of all the genes around the Rrs1 loci a mapping population comprising >10,000 F2 from the cross SBCC145 x Beatrix has been constructed. F2 screening of about 10,000 lines to select recombinant lines between two flanking markers has identified around 385 verified recombinant plants. The development of diagnostic markers for Rrs1 will be the basis to incorporate scald resistance in modern barley varieties and

the haplotype analysis will help to get more information to discuss the evolution of the Rrs1 locus.

Impact of meiotic crossover and gene conversion on the genomic landscape of Arabidopsis recombinants

Korbinian Schneeberger

Max Planck Institute for Plant Breeding Research, Genome Plasticity and Computational genetics, 50829 Cologne, Germany

Knowledge of the exact distribution of meiotic crossovers (COs) and gene conversions (GCs) is essential for understanding many aspects of population genetics and evolution, from haplotype structure and long-distance genetic linkage to the generation of new allelic variants of genes.

We resequenced the four products of 13 meiotic tetrads along with 10 doubled haploids derived from *Arabidopsis thaliana* hybrids. However, identification of GC based on resequencing data can be severely confounded by genomic rearrangements and need to account for non-allelic, homologous sequence. For example, rigid filtering for structural variation and mis-placed reads during resequencing revealed an ~80-kb transposition, which underwent copy-number changes mediated by meiotic recombination.

Non-crossover associated GCs were extremely rare most likely due to their short average length of ~25–50 bp, which is significantly shorter than the length of CO-associated GCs. Overall, recombination preferentially targeted non-methylated nucleosome-free regions at gene promoters, which showed significant enrichment of two sequence motifs.

Towards cloning t*hresh-1*, a domestication gene causing a difficult to thresh phenotype in wild barley germplasm

Stefanie Pencs[1], Vera Draba[1], Rachel Burton[2], Geoff Fincher[2], Nils Stein[3], Klaus Pillen[1]

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Barley seeds that show an increased solidity of palea and awns have an advantage for seed dispersalin nature. However, under agricultural conditions they turn out to be difficult to thresh, which results in problems, especially during mechanical seed drilling. The exotic *thresh-1* allele, controlling grain threshability, was identified in the wild barley introgression line S42IL-143, which is derived from the *H. vulgare* ssp. *spontaneum* accession ISR42-8. The locus *thresh-1* was mapped to chromosome 1H within a 4.3cM interval (Schmalenbach et al. 2011). Barley seeds possessing the recessive *thresh-1* allele exhibit a difficult to thresh phenotype, probably due to the action of a gene, which is involved in cell wall composition. The aim of our current project is to identify and clone candidate genes for the *thresh-1* locus.

To accomplish this, we first increased the genetic resolution of the *thresh-1* region by mapping threshability in the barley nested association mapping (NAM) population HEB-25. The population consists of 1,420 BC_1S_3 lines, originating from crosses of the spring barley cultivar Barke with 25 wild barley donor accessions. HEB-25 has been genotyped with the 9k barley iSELECT chip (Pillen pers. comm.). *Thresh-1* was mapped in HEB-25 to the same region on chromosome 1H as in S42IL-143. We could place the gene between two flanking SNPs revealing 0cM distance in the reference map of Comadran et al. (2012). Based on the genotype data we currently select informative recombinants within a target interval of 5cM around the *thresh-1* locus and build two pools (easy to thresh versus difficult to thresh) based on phenotype data from three consecutive growing seasons. These pools will be subjected to exome capture analysis in order to identify candidate genes, which co-segregate with the threshability phenotype. Finally, the identified candidate genes will be subjected to complementation experiments in order to validate the true nature of *thresh-1*.

In parallel to the genetic analysis, biochemical tests were conducted with the original introgression line to search for differences in cell wall composition between difficult and easy to thresh genotypes. First results indicate that the introgression line S42IL-143 and the control genotype Scarlett exhibit different cellulose and mixed linkage β-glucan contents in leaf tissues as well as in palea and awn tissues.

Identification of epistatic effects in a barley MAGIC population -
understanding phenotypic variation in complex traits

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Determining the genetic architecture of complex traits is a necessary step to understand phenotypic changes in natural and experimental populations. However, this is still a major challenge for modern genetics, since the estimation of genetic effects tends to be complicated by epistasis, which leads to changes in the effect of allelic substitutions depending on the genetic background (Le Rouzic). Epistatic effects are statistically defined as interactions on a phenotype between effects of alleles from two or more genetic loci which do not correspond to the sum of their separate effects (Fisher, 1918). Studies in self-pollinated crops have been successful in given evidence for important epistatic effects, for example concerning yield or flowering time in barley (von Korff et al., 2010). The power to detect epistatic effects not only depends on the trait of interest but as well on the mapping population and the statistical method used. The barley multi-parent advanced generation intercross (MAGIC) DH lines derived from eight parents proofed to be a valuable resource in mapping QTL of complex traits like flowering time. Therefore, epistatic effects for the trait “days to heading” were estimated using a newly developed mixed linear model in SAS 9.2, incorporating multi-locus analysis and cross-validation. The results enabled the comparison of the epistatic effects with the standard single SNP binary approach (BA), widely used in QTL and association mapping with a haplotype approach (HA). The detected epistatic effects with the haplotype approach allowed an almost entire genetic explanation of the phenotypic variation.

Fisher R.A. (1918) The correlation between relatives on the supposition of mendalien inheritance. Transaction of the Royal Society of Endinburgh 52:399-433
Le Rouzic A., Álvarez-Castro J.M., Carlborg Ö. (2008) Dissection of the genetic architecture of body weight in chicken reveals the impact of epistasis on domestication traits. Genetics 179:1591-1599
von Korff M., Leon J., Pillen K. (2010) Detection of epistatic interactions between exotic alleles introgressed from wild barley (H. vulgare ssp. spontaneum). Theoretical and Applied Genetics 121:1455-1464. DOI: DOI 10.1007/s00122-010-1401-y.

An Update on the Sugar Beet Genome Sequence and its Annotation

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Sugar beet (Beta vulgaris ssp. vulgaris) is a diploid plant with n=9 chromosomes and an estimated genome size of 714-758 Mbp. The taxonomical position of the species is within the Amaranthaceae family and in the order Caryophyllales. The plant is an important crop in Europe and the US, accounting for 30% of the yearly global sugar production. While beet varieties cultivated for their leaves have been known since Roman times, sugar beet is one of the most recently domesticated crops. First efforts of breeding sugar beets date back to the end of the 18th century, following the discovery that the sugar accumulating within the beet's storage root is chemically identical to sugar extracted from sugar cane.
We sequenced the sugar beet genome and generated an assembly that encompasses 567 Mbp, of which 85% are chromosomally assigned. This genome sequence is the first representative from the Caryophyllales clade, a taxon comprising 11,500 species. In addition to Beta vulgaris and Spinacia oleracea, this clade also includes species such as the cacti, stone plants, and several carnivorous taxa. It has been shown previously that beets share an ancient genome triplication with other eudicot plants. We now constructed phylogenetic trees for each sugar beet gene (collectively referred to as "phylome") and used the data to infer accurate phlyogenies among eudicot plants. Our analysis revealed the separation of Caryophyllales prior to the split of rosids and asterids. The genome contains about 27,500 protein-coding genes that were predicted with the support of transcript data. Sugar beet contains less genes encoding transcription factors than any other taxon included in our analysis (five rosids, two asterids, two monocots).
The sugar beet genome sequence paves the way for further molecular and comparative studies in sugar beet, Caryophyllales and eudicot plants.

The humble potato goes globe-trotting: The escape from day-length regulation of potato tuberisation

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Potato reproductive development is regulated by the sensing of environmental cues resulting in the activation of a signalling cascade. Signal molecules synthesised in the perceiving organs are transported to the cells and tissues that go on to develop into flowers or tubers. Although soil nutrients and factors such as water availability and ambient temperature are important signals for reproductive development, the major environmental component for tuberisation is day-length. Transition from long to short days also influences important agronomic traits such as earliness and maturity. A major-effect QTL for Plant Maturity and Earliness in potato has been mapped on Chromosome 5 in diploid clones and in tetraploid material. Using map based cloning together with deep bi-allelic sequencing of chromosome 5, we have identified a gene coding for a central regulator underlying this major-effect QTL. We show that this gene, named StCDF1, belongs to the family of DOF transcription factors that regulates tuberisation and plant life-cycle length, by acting as a mediator between the circadian clock and the mobile tuberisation signal StSP6A. We have shown that naturally occurring allelic variants of this protein, evade post-translational regulation, thereby leading to early tuber formation and shortened life-cycle. We propose that this allelic variation has allowed the cultivation of potato outside the Andean centre of origin, under long-day conditions of spring and summer that prevail during the growing season in northern temperate latitudes.

The circadian clock and photoperiodic flowering in barley

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Seasonal changes in photoperiod are important developmental cues in barley. The major photoperiod response gene *Ppd-H1* accelerates flowering in response to long day, but not short day conditions in barley. We have shown that mutations in the clock genes *HvELF3* and *HvLUX1*, underlying the early maturity loci *eam8* and *eam10,* respectively*,* cause photoperiod insensitivity and early flowering under long day and non-inductive short day conditions (Faure et al. 2012; Campoli et al. 2013). Mutations in *HvELF3* and *HvLUX1* both altered clock function and caused early flowering by up-regulating *Ppd-H1* in barley. This increased expression of *Ppd-H1* in *eam8* and *eam10* suggests a conserved function between Arabidopsis and barley; in Arabidopsis related members of the PRR family are primary targets of *ELF3* and *LUX*. These results suggest that the circadian clock plays an important role in photoperiod control in barley. Interestingly, mutations in *HvELF3* were used to breed barley varieties adapted to short growing seasons, although these lines are severely compromised in clock function. In contrast to barley clock mutants, variation in the barley clock did not have strong effects on growth and primary assimilation.

The sex-linked region in Populus tremuloides Turesson 141 corresponds to a pericentromeric region of about two million bp on P. trichocarpa chromosome 19

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In the dioecious genus Populus, sex determination has been located to chromosome 19. But, despite a high degree of genome collinearity, various Populus species seem to differ in the location of the sex-determining region on the respective chromosome and in the apparent heterogametic sex. In contrast to Populus trichocarpa, where sex determination was reported to be located in a peritelomeric position on chromosome 19 with femaleheterogamety (WZ-system; Yin et al. 2008), the sex determining region in P. tremuloides was shown to be located on a central position with male heterogamety (XY system; Pakull et al. 2009, 2011). Sequence characteristics of two BAC-clones representing parts of the sex-linked region from P.tremuloides as well as the P. trichocarpa region including the markers sex-linked in P. tremuloides indicate that the sex-linked region in P. tremuloides is pericentromeric (Kersten et al. 2012).The boundaries of the sex-linked region in P. tremuloides Turesson 141 were defined using SSR and SNP markers (Pakull et al. 2011, Kersten et al. 2013). Markers were genetically mapped in an interspecific P. tremula L. (Brauna 11) x P. tremuloides Michx. (Turesson 141) cross (118 F1 individuals). The physically corresponding P. trichocarpa region was screened for candidate genes for sex determination based on extended functional annotation using the Mercator annotation pipeline(http://mapman.gabipd.org/web/guest/app/mercator) and Blast2GO (http://www.blast2go.com/).The boundaries of the sex-linked region on the male P. tremuloides map were defined each by a pair of markers located in relative physical proximity to each other, of which one marker was completely sex-linked and the other showed a single individual deviating from full sex-linkage. The markers that define the boundaries so far span about 2 million base pairs on a central region of the P. trichocarpa chromosome 19 (Kersten et al., 2013). This region represents a region of recombination suppression. Since centromeres are generally considered as regions of low recombination, this could support the theory of a pericentromeric location of the sex-linked region in P. tremuloides (Kersten et al. 2012). A co-localisation of sex-determining regions and regions of already suppressed recombination such as, e.g., centromeres, has also been reported in other species, e.g. in papaya (Liu et al. 2004).The region of P. trichocarpa chromosome 19 (Tuskan et al. 2006) corresponding to the region sex-linked in P. tremuloides comprises 65 gene loci with 135 transcript models (Phytozome, v3.0 annotation). Since sex determination in Populus has been reported to occur before the initiation of stamen and carpels, and regularly only organs of a single sex are initiated in Populus flowers (Brunner 2010), we were interested in genes putatively involved in early steps of flower development as well as in transcription factors which have been shown to be involved in sex determination in some non-plant species. Seven potential candidate genes for sex-determination were selected based on these criteria (Kersten et al. 2013).Nevertheless, despite high genome collinearity, P. trichocarpa is another species, with reported differences in the location of the sex-determining region and the apparent heterogamentic sex. For that reason the sex-determining gene(s) in P. tremuloides may not be present in the accordant P. trichocarpa genome region at all. The exploration of P. tremuloides genomic sequence data is essential for a final search for candidate genes. NextGeneration sequence data of male and female P. tremuloides individuals were

generated recently. The bioinformatic analysis aiming to identify molecular markers to differentiate between both sexes early in plant development is under way.

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Developing and exploiting genomics tools for barley improvement and gene discovery

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Developing genomics tools for barley has resulted in the improved ability to exploit germplasm resources for barley breeding and gene discovery. The Triticeae Coordinated Agricultural Project (TCAP) is a US-based project focused on developing and utilizing genomics tools to identify quantitative trait loci associated with climate change traits. The ultimate goal is to use the information in molecular breeding approaches to improve wheat and barley. In the TCAP, key barley germplasm resources being used are the National Small Grain Core Collection (NSGCC) and the wild barley introgression population (WBIP). The NSGCC consists of 1860 unique accessions (landraces, varieties, and genetic stocks) from approximately 100 countries. The NSGCC was genotyped with 6,224 SNPs and phenotyped for numerous agronomic (e.g., yield, heading date, height, and tillering), and disease resistance (e.g., spot form net blotch, and stem rust) traits. The WBIP (792 individuals) was developed by conducting two backcrosses of an elite six-row spring barley cultivar Rasmusson with 25 wild barley accessions. This population was genotyped with 384 SNPs and 4,200 SNPs were imputed onto the population. The WBIP was also phenotyped for numerous agronomic traits including: heading date, height, and yield. For both the NSGCC and the WBIP, the combined genotype and phenotype data was used to conduct genome-wide association studies resulting in the identification of numerous QTL. In addition to identifying QTL for barley improvement, genomics resources also provide an efficient avenue for isolating barley genes. Tillering is an important agronomic trait that impacts overall grain yield. The *uniculm2* mutant does not tiller due to a disruption in axillary meristem development. Mutagenesis of the *uniculm2* mutant resulted in the identification of two mutants that suppress the *cul2* mutant phenotype resulting in *cul2* homozygous mutants that tiller. These mutants are referred to as *suppressor of cul2-1* and *-2* (*scu2-1 and scu2-2*). RNA-seq coupled with genetic mapping identified the underlying gene. Interestingly, the protein has no known function but is highly conserved in plants. A discussion of the role of the *scu2* gene will be presented. Taken together, these examples highlight the power of coupling genomics tools with breeding and gene discovery.

The wheat genome sequence: 17 Gb of opportunities for wheat improvement

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In 2005, a group of growers, breeders, and plant scientists launched the International Wheat Genome Sequencing Consortium (IWGSC) with the goal of securing a high quality, reference sequence of the bread wheat genome. A milestone-based strategy coupled with short and long-term roadmaps was established to provide breeders access to an increasing array of tools and resources. To reduce the complexity of the hexaploid, highly repetitive, 17Gb bread wheat genome, the IWGSC follows a chromosome-specific approach. The strategy to obtain a high quality reference sequence anchored to the genetic and phenotypic maps consists of 5 main steps: (1) isolation of individual chromosomes or chromosome arms by laser flow cytometry and construction of BAC libraries for each of the 21 wheat chromosomes, (2) fingerprinting and assembly of physical maps anchored to genetic maps with molecular markers, (3) sequencing of the Minimal Tiling Path (MTP) for each chromosome and scaffold assembly, (4) construction of a pseudomolecule through the ordering of the sequence scaffolds along the chromosome and (5) annotation of the pseudomolecule.

The first milestone and proof of concept was achieved in 2008 with the construction of the physical map of chromosome 3B. A MTP of 8,452 clones was defined and used for sequencing using a NGS hybrid approach. Using SNP markers derived from each scaffold and a combination of genetic and LD mapping, a pseudomolecule of 774 Mb has been achieved. Annotated using the TriAnnot pipeline identified 7,264 protein-coding genes and 85% of transposable elements. Distribution of structural and functional features along the chromosome revealed a striking compartmentalization likely driven by meiotic recombination and the preferential selection of genes involved in adaptation in the most distal and dynamic regions of the chromosome. Comparative analyses with model grasses revealed massive and recent superimposed inter- and intra-chromosomal gene duplications that provide new sources of functional adaption in wheat.

To date, physical maps have been completed or are underway for all 21 chromosomes, and MTP sequencing has started for a few other chromosomes. To facilitate anchoring, marker development, and gain a first insight into the gene space composition and evolution of the wheat genome, the IWGSC recently completed the shotgun sequencing of each of the 21 chromosomes (CSS). Annotation of sequence contigs revealed 124,201 high-confidence protein-coding genes distributed nearly equally across the three subgenomes and provided for the first time access to the gene content and expression of each chromosome. Comparative analyses with diploid and tetraploid relatives shotgun genome sequences indicated that polyploidization was not followed by massive gene loss on the subgenomes and mostly affected large gene families. Transcriptome studies did not show any global dominance of a subgenome but indicated extensive subfunctionalisation. By enabling access to the information for each individual chromosome, the CSS provides a new foundation for the specific design of molecular markers and of exome capture assays thereby greatly facilitating the development of efficient molecular tools for breeders.

Population genomic and transcriptomic analysis of local adaptation in wild barley

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Crop wild relatives are considered to be a valuable genetic resource for crop plants. While the introgression of resistance genes from exotic germ plasm into elite cereal varieties has been achieved numerous times, the introgression of traits based on multiple genes is more challenging. We explore the potential of wild barley to serve as a source of useful genetic variation for adaptation to abiotic stress by characterizing genes involved in evolutionary adaptation to extreme environments. Since the reproductive stage is particularly sensitive to drought, the analysis of drought tolerance genes affecting reproductive success contributes to understanding adaptive mechanisms. We used an ecological genomics approach to investigate the reproductive response of drought-tolerant and sensitive wild barley accessions originating from different habitats in the Levant region. Differentially adapted genotypes were identified from a larger set of accessions using repeated drought stress trials in the greenhouse. Messenger RNA from spikelets at the flowering stage from drought-treated and control plants was Illumina sequenced and aligned to the cultivated barley reference genome sequence database and the expression level measured. This approach enabled the detection of biological processes affecting grain production under drought stress. We identified novel candidate genes and differentially expressed alleles associated with drought tolerance. Drought associated genes were shown to be more conserved than non- associated genes, and drought-tolerance genes were found to evolve more rapidly than other drought associated genes. The analyses revealed that reproductive success after drought stress is not a habitat-specific trait but a shared physiological adaptation of genetically distinct barley accessions. Further analyses will dissect the genetic basis of this physiological adaptation.

Natural variation in circadian rhythms suggests an effect of domestication on the circadian clock of tomato

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The circadian clock controls many important aspects of plant physiology and development, including several traits of agronomical significance in crop plants. In addition, natural variation in circadian rhythms appears to be important for adaptation to specific environments. Still, quantitative differences in circadian rhythms due to artificial selection have not yet been reported. By monitoring circadian leaf movements and transcript rhythms, we found that cultivated tomato (*Solanum lycopersicum*) exhibits different rhythms than its wild relatives, indicating that domestication or early breeding has had an effect on the tomato circadian clock. Quantitative trait locus (QTL) analysis in two populations derived from crosses between cultivated and wild tomato accessions identified one locus controlling differences in circadian rhythms. Fine mapping of this locus resulted in a region containing 13 candidate genes. Near isogenic lines differing in those 13 genes exhibit differences in seedling growth in the greenhouse indicating that variation in circadian rhythms could be changing plant growth under diurnal conditions. In conclusion, our work suggests that humans may have selected for altered circadian rhythms during tomato domestication or early breeding to adapt the species to agricultural environments.

Towards engineering C4 photosynthesis: Lessons from transcriptomic and evolutionary analyses

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One of the limitations on crop productivity is the efficiency with which inorganic carbon is converted to carbohydrates in photosynthesis. Carbon conversion efficiency, in addition to light harvesting and conversion and the harvest index is thus a prime target for engineering highly efficient crops. Carbon conversion efficiency is constrained by the biochemical properties of the carbon-assimilating enzyme RubisCO, which accepts both carbon dioxide and molecular oxygen as substrates for the carboxylation or oxygenation of ribulose 1,5-bisphosphate, respectively. The oxygenation reaction reduces carbon conversion efficiency and requires the process of photorespiration to detoxify the product of the oxygenation reaction, 2-phosphoglycolate. Cyanobacteria, algae, and some land plants have evolved mechanisms to concentrate carbon dioxide at the site of RubisCO, thereby reducing the magnitude of oxygenation and hence increasing the conversion efficiency. In land plants, C4 photosynthesis is the predominant carbon concentrating mechanism that has convergently and concurrently evolved over 60 times in monocotyledonous and dicotyledonous flowering plants. Some of our most efficient crops, such as corn and sugarcane are C4 plants; however, others, such as rice and wheat are C3 plants that display relatively low carbon conversion efficiency.
To uncover the genetic architecture of the C4 trait, we are employing transcriptomic comparisons of closely related C3 and C4 species to identify those genes that are differently expressed between C3 and C4. Through multivariate statistics, we identify transcriptional regulons constituting the trait and through metabolic modeling we unravel the evolutionary trajectories leading from C3 to C4 via intermediate states of C2 photosynthesis. Eventually, this work will provide the blueprint for the reconstruction of C4 in a C3 background, using the tools provided by synthetic biology.
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Haploid and transformation technology in cereals

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The generation and use of doubled haploids (DHs) is one of the most powerful biotechnological means to improve cultivated plants. The biological phenomenon most widely employed in haploid technology is that immature pollen grains are capable of turning their fate towards embryonic development instead of continuing the regular process of pollen maturation. As a result, the random meiotic recombination of the mother plant's genetic make-up is embodied in populations of individually distinct, yet perfectly true-breeding (genetically fixed) DH lines, provided that a spontaneous or artificially triggered duplication of the haploid genome gives rise to diploid cells that contribute to the germline. DH populations enable the breeder to select with high precision and efficiency with regard to any trait or trait complex. However, the use of pollen embryogenesis is associated with limitations such as recalcitrance of some species, genotype dependency, albino formation as well as unbalanced occurence of the theoretically possible genetic recombinants. Furthermore, the improvement of pollen embryogenesis is essentially hampered by our poor understanding of the underlaying biological processes. As a consequence, we have been taking an integrated research approach that encompasses ultrastructural studies, live-cell imaging as well as comparative transcript, protein and metabolite profiling using barley as experimental model. The long-term objective of these investigations is to provide a fundament for the knowledge-based development of powerful haploid technology implemented in plant breeding and applied research. An example for the latter is the emerging field of genome engineering, where designer-endonucleases are used to obtain site-directed genetic modifications *in planta*. With the aim to establish genome engineering in cereals, we expressed *GFP*-specific transcription activator-like effector nucleases (TALENs) in embryogenic pollen cultures of barley harboring the *GFP* gene as stably integrated target sequence. Thanks to the haploid nature of the host cells and the screenable marker gene used, knock-out mutations were readily detected and, following genome duplication, homozygous primary mutant plants obtained. Over 20% of the TALEN transgenics proved knocked out with respect to *GFP*, and the loss of function could be ascribed to deletions of between 4 and 36 nucleotides in length. The altered *GFP* alleles were transmitted normally through meiosis, and the knock-out phenotype was consistently shown by the offspring of two independent mutants. In the progeny of another primary mutant, additional *GFP* alleles were observed that had not been detected in the parental plant. From the data obtained, we infer various scenarios of the formation of homozygous, bi- and multi-allelic as well as chimeric mutants in dependence on host cell ploidy, mutation of TALEN binding sites, mutant cell contribution to the germline and generative segregation of alleles. Site-directed genome engineering is a breakthrough technology that will greatly facilitate the functional validation of genes. Likewise, it offers versatile novel possibilities of crop improvement. In this context it is particularly remarkable that TALENs act as mutagen *in planta* rather than giving rise to recombinant DNA. Consequently, plants carrying TALEN-induced small genetic alterations may not necessarily fall under the European (over)regulation of genetically engineered organisms, on the condition that any TALEN-coding expression cassettes integrated in the host genome are removed by segregation. In addition, our results may also pave the way

for the establishment of even more sophisticated procedures using customizable endonucleases to precisely edit cereal and other crop plant genomes based upon double strand break repair *via* homologous recombination.

Subgenome-specific expression analysis of Fusarium-responsive genes in *Triticum aestivum*

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Transcriptomic analyses of wheat near-isogenic lines (NILs) segregating for prominent resistance QTL have not yet yielded the causal genes for either Fhb1 or Qfhs.ifa-5A. The yet still missing complete genome annotation of wheat presents a major drawback for such experiments. To date the most complete assembly of the T. aestivum gene space is described by the released wheat low-copy-number genome (LCG) assembly, which provides partial sequences of an estimated 94 k genes. In addition, the annotation of the close relatives (e.g. Aegilops Tauschii or Hordeum vulgare) may be used. However these gene models are either incomplete and lack detailed annotations or do not differentiate between homeoalleles. The IWGSC (International Wheat Genome Sequencing Consortium) has far advanced in the generation of a whole genome map of wheat. We have used these preliminary data as reference for a recent RNA-seq experiment that captured the response of NILs to Fusarium graminearum (Kugler et al. 2013). Mapping transcripts that show differences in the Fusarium response between lines harboring the resistant or susceptible alleles of either Fhb1 or Qfhs.ifa-5A highlights several transcripts onto the respective regions of the QTL on chromosomes 3BS and 5A. These genes will be selected for further analyses.

Identification of Intervarietal Substitution Lines of Rapeseed with Enhanced Embryogenic Potential

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In many Brassica species it is possible to induce a sporophytic development in isolated microspores, which first leads to the formation of "microspore derived embryos" (MDEs) that can be further developed into doubled haploid (DH) lines. Today microspore embryogenesis and DH lines are of great importance in practical breeding and basic research. The main limiting factor in microspore embryogenesis is the embryogenic potential of the microspores, that is the frequency, with which the microspores form embryos. In rapeseed the embryogenic potential varies strongly between genotypes, but not much is known about its genetic control. In DH mapping populations, alleles causing differences in embryogenic potential are supposed to lead to skewed segregations at markers linked to the loci where the alleles are segregating. To localize genetic factors controlling embryogenic potential in rapeseed, a genetic map of 481 AFLP markers was constructed in a population of 191 MDEs derived from one F1 plant of the cross 'Express 617' x 'RS239'. A total of 236 markers in 33 regions showed significant deviation ($P< 0.05$) from the expected segregation ratio. Five regions, which showed regular marker segregations in a corresponding BC1 population, were chosen and 15 intervarietal substitution lines (ISLs) from the same cross with 'Express 617' as recurrent parent with donor segments in these regions were selected. The embryogenic potential of the ISLs was determined and compared to the potential of 'Express 617'. Seven ISLs showed a significantly ($P< 0.02$) enhanced embryogenic potential, ranging

Massive Analysis of cDNA Ends (MACE), a reduced complexity high-throughput transcriptome analysis method for simultaneous gene expression analysis and genomic mapping (TranSNiPtomics) in species with known and unknown genomes

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High-throughput sequencing based transcriptome analysis is becoming a standard tool for SNP discovery and gene expression analysis. However, very deep sequencing is required in order to obtain sufficient coverage for reliable SNP detection and quantification. This concerns especially rare transcripts, like for example from transcription factors and resistance genes. Here we present an improved version of 3' UTR sequencing coined "Massive Analysis of cDNA Ends" that tackles this problem by sequencing only one single fragment per transcript molecule, originating from the 3' or 5' end of the transcript. Hence, each transcript molecule is represented by only one single read, reducing the required sequencing depth for reliable gene expression analysis to one 20th when compared to RNAseq. As only about one 20th of the sequence of each transcript is sequenced, the coverage of the sequenced area is drastically improved compared to RNAseq at similar sequencing depth and SNPs can be discovered more efficiently. The technique is combined with a method to eliminate PCR bias (TrueQuant) which guarantees reliable quantification and better SNP discovery, as PCR-copies containing pseudo-SNPs are eliminated from the dataset. Here we present MACE analyses for the efficient detection of a BYDV resistance gene in Barley and for stem rust disease in Lolium as well as for the analysis of a drought resistance QTL in *Cicer arietinum*.

Quantitative analysis of post-translational modifications in the Arabidopsis proteome.

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Post-translational modifications (PTM) play a key role in cellular signaling and regulation of metabolic pathways. They provide a powerful mechanism to rapidly and temporarily alter protein functions and locations within the cell, and they are also capable of providing information to regulatory proteins by creating docking sites for PTM-recognition domains. Several thousands of proteins are known to be post-translationally modified within a plant cell, however in most cases it is not known whether these sites are regulated and thus important for the regulation of the function of the protein. Hence the development of new techniques in quantitative proteomics are of great importance. This talk focusses on new techniques for the enrichment and quantification of PTM-sites on proteins and examples for the quantitative analysis of lysine acetylation sites will be presented.

Discovery of diagnostic DNA markers for complex agronomic traits of potato by combining comparative proteomics/transcriptomics with association genetics

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Most characters relevant for commercially viable potato varieties are complex, meaning that they are controlled by multiple genetic and environmental factors. Examples of such complex traits are tuber yield and starch content, tuber reducing sugar content (determining processing quality), tuber susceptibility to bruising and quantitative resistance to late blight caused by *Phytophthora infestans*. DNA based markers useful for marker-assisted selection in tetraploid potato are expected to be diagnostic for the trait of interest in multiple, heterozygous genetic backgrounds, in other words, the marker should be in strong linkage disequilibrium with superior or inferior alleles of the trait of interest. Ideally, diagnostic markers are derived from the genes that directly control the trait. With the objective to identify such diagnostic markers de novo, we quantitatively compared protein and transcript profiles of tetraploid genotypes that have been selected for contrasting phenotypic values such as high versus low tuber susceptibility to bruising, tuber reducing sugar content or resistance to late blight (cases and controls). This resulted in a number of novel differential proteins and transcripts that were not known so far as having a functional role in these traits. DNA polymorphisms in the corresponding genes were subsequently analysed for association with positive or negative trait values in populations of tetraploid varieties and breeding clones that have been phenotyped by breeders for the respective traits. Highly significant marker-trait associations were identified, examples of which are presented.

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Mining QTL for candidate genes involved in resistance of oilseed rape against Verticillium longisporum by an integrative omics approach

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Verticillium longisporum is an increasing threat to winter oilseed rape production in Europe. Resistance against V. longisporum is quantitatively inherited. A mapping population produced from an inbred line of a German rapeseed cultivar and a resynthesized rapeseed line segregating for V. longisporum resistance (Express617 x R53) was used in a combination of a non-targeted and a targeted genetic, genomic, transcriptomic and metabolic analysis approach for the development of molecular markers. The non-targeted analysis included the production of a high density genetic map using genotyping-by-sequencing and quantitative trait locus (QTL) mapping for disease related traits. The targeted analysis included the identification and quantification of phenolic metabolites and lignin monomers in the hypocotyls of the mapping population to evaluate association with resistance expression and identify metabolite QTL co-localizing with resistance QTL. Integration of metabolomic and genetic data from the mapping population with transcriptomic data obtained by RNA-Seq analysis from contrasting genotypes allowed to mine for pathways and candidate genes with a high contribution to resistance expression. Molecular markers are being derived and validated for their usefulness in marker-assisted selection for plant breeding.

"A walk on the *Omics* side …" – An integrative approach to investigate iron deficiency associated chlorosis in Maize.

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Improving nutrient homeostasis is a major challenge of a sustainable plant cultivation, and cornerstone to ensure food supply for a growing world population. Although, iron constitutes an important nutrient, iron availability is limited. In this respect, iron deficiency associated chlorosis in maize and other plant species causes severe yield losses every year.

Iron deficiency can be considered as a complex trait that is influenced by a multitude of molecular components. These act together in specific environments and shape plant performance. Natural variation of the iron deficiency response in maize has been addressed by an integrative *Omics* approach in order to identify contributing genes and pathways. Correspondingly, combination of i) genomic proceedings within the maize intermated B73 x Mo17 population , ii) transcriptome profiling by RNA-Seq and qRT-PCR as well as iii) identification and quantification of proteins by LC-MS/MS enabled us to link multiple layers of molecular information. In consequence, the impact of already known components involved in iron homeostasis could be substantiated (e.g. Methionine salvage pathway, bHLH regulators), and extended by some novel twists. Furthermore, new candidate genes and molecular concepts contributing to differential iron deficiency associated chlorosis were identified.

Finally, this study can be considered as conceptual design to investigate complex crop traits by "integrative *Omics*" in order to foster information transfer between basic research and applied science and promote application development.

proWeizen – the German Wheat Research and Breeding Alliance

Tanja Gerjets, proWeizen c/o Gesellschaft für Erwerb und Verwertung von Schutzrechten GVS mbH, 53115 Bonn, Germany

Wheat is one of the world's most important crops and Germany is one of the most important wheat producers. In recent years, yield improvements in wheat lagged behind those observed in other major crops. To overcome this, it is necessary to both join and increase efforts in wheat breeding and research. Realizing this, the German wheat breeders have recently founded the German Wheat Research and Breeding Alliance proWeizen to combine the scientific excellence in wheat research and the breeding expertise in Germany. proWeizen offers support in project management, liaises between wheat breeding and research on national and international level, participates in national and international efforts of wheat research and breeding and helps with mobilizing of funding opportunities. In spring 2013, the Federal Ministry of Food, Agriculture and Consumer Protection announced a call for wheat research projects focusing on breeding for yield increase and stability, better adaptation to environmental stresses and utilisation of heterosis. The proWeizen platform is equally open to scientists and companies working in wheat breeding and research.

POSTER SESSION

poster number 1

Breeding of Russian dandelion (Taraxacum koksaghyz) – From the wild type to a new resource for a sustainable rubber production

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The property of Russian dandelion (Ta-raxacum koksaghyz) to store high quali-ty rubber in its roots has been known for a long time but breeding or even industrial production has not been per-formed consequently since efforts dur-ing World War II. Now, in the light of an increasing demand for natural rubber and lack of alternatives to the Para rub-ber tree (Hevea brasiliensis), rubber production of Russian dandelion shall be enhanced and provide a new sus-tainable resource for the natural rubber demanding industries.
Due to the high diversity and relatively weak growth of T. koksaghyz, it is still considered a wild type. This work aims to gain insights into the genetic back-ground of this plant and provide important information for promising breeding programs, in order to get the Russian dandelion on its way to a seri-ous rubber producing crop.
In a network of different research insti-tutions and in close cooperation with a breeding partner, the comprehensive genetic variability of T. koksaghyz shall be used for the development of new varieties with high level and quality of rubber. On that account, different ag-ronomic traits, such as the formation of a large, clear taproot with high contents in rubber and inulin, early and uniform flowering time, improved tillering in the first year of cultivation, as well as dif-ferent disease resistances, have been defined as breeding objectives.
These objectives will be supported by (I) drafting a dendrogram of different Taraxacum species based on AFLP anal-ysis for information on the genetic background of T. koksaghyz and identification of hybrids resulting from inter-specific crosses, (II) development of a genetic map of T. koksaghyz as basis for the development of selection markers and (III) a marker-assisted advanced backcross program. These work pack-ages are focused on providing infor-mation and genetic tools for marker-assisted breeding of T. koksaghyz as a new commercial rubber crop.

poster number 2

Identification of QTLs for drought stress tolerance and drought stress induced leaf senescence using wild barley introgression lines

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Fifty-two wild barley introgression lines (ILs) were screened for their drought stress response at an early developmental stage. The introgression lines of the population "S42IL" are derived from a cross between the malting barley cultivar "Scarlett" and the wild barley accession "ISR42-8". The goal of the project was to identify genotypes that differ in their performance from the cultivar parent "Scarlett", and to identify QTLs for drought stress related traits.
Four experiments were conducted at the experimental station "Kühnfeld" at the Martin-Luther-University in Halle in spring and autumn 2011. The introgression lines and the cultivars "Scarlett", "Marthe" and "Barke" were grown for one month under greenhouse conditions. The trials were carried out with three replications under two water treatments –drought stressed and control. On the day of sowing all pots were irrigated with 400 mL of water. One week after germination automatic watering of control plants by mat irrigation started. Plants under drought treatment did not receive any additional water. During the experiment leaf greenness (SPAD) and photo system II efficiency (PSII) (Mini-PAM, Walz) were assessed on the primary leaves in order to detect drought stress induced leaf senescence. At completion of the experiment fresh biomass (BMF) and dry biomass (BMD) as well as tiller number (TIL) and plant height (HEI) were determined.
Across four experiments we identified 23 genetic effects among 13 "S42ILs" for the traits BMD, TIL, HEI and PSII as main effects or as line by treatment interaction effects. Some lines showed the same effect under both treatments. Therefore the 23 effects were summarized to 14 QTLs.
With the 13 ILs that showed effects across the experiments we performed two verification experiments in spring 2012. The number of replications was increased to six and more space per pot was allowed.
The QTL-Analysis revealed effects for all six measured traits. Stress indices were calculated for all traits. In total 47 genetic effects across treatments or as line by treatment interaction effects were discovered. Some effects were detected as main effect as well as line by treatment interaction effects. Those were summarized to one QTL. A total of 28 QTLs were found for the six traits and three stress indices. Of the 14 QTLs discovered in 2011 we verified nine QTLs. 19 QTLs were new QTLs. Eleven of the 28 QTLs were also detected in previous studies on the S42- and the S42IL-population.

poster number 3

Identification of candidate genes for a BaYMV/BaYMV-2 resistance gene located on barley chromosome 5H

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Barley yellow mosaic virus disease, transmitted via the plasmodiophorid Polymyxa graminis, is caused by different strains of soil-borne Barley yellow mosaic virus (BaYMV) and Barley mild mosaic virus (BaMMV) and is one of the most important diseases of winter barley in Europe and Asia. Chemical measures to ensure barley production in the growing area of infested field are neither effective nor acceptable for ecological reasons. Therefore breeding for resistance is the only way to avoid high yield losses. Several loci conferring resistance to the different strains of BaMMV and BaYMV are known. Recently a new gene was discovered being only effective against BaYMV and BaYMV-2 located on chromosome 5H. A map based cloning approach was started in order to get information on the structure and function of this gene. 5085 F2 plants corresponding to a resolution of 0.0098% recombination derived from the cross 'HHOR4224' x 'Igri' were examined with co-dominant flanking markers to construct a high resolution mapping population. The target interval carrying the resistance locus was estimated at 12.08 % recombination in this population. Marker saturation of this target interval is conducted by using all marker and sequence information available in barley, and employing synteny to rice, sorghum, Brachypodium and newly developed sequence information of barley included in the genome zipper. Up to now, 23 additional markers have been mapped in the gene carrying interval. Besides this, out of 707 segmental recombinant inbreed lines identified, 375 RILs were tested on two locations for BaYMV/BaYMV-2 resistance. The segregation ratio observed revealed a good fit to a segregation ratio of 1r:1s (X2=0.52). Based on these data and the newly developed markers the BaYMV/BaYMV-2 resistance gene was located between markers 1_xxx1 and Bradi4gxx2 in an interval of 0.30 % recombination. Further marker saturation will be conducted using an exome capture sequencing approach.

poster number 4

Genome-wide analysis of population structure and linkage disequilibrium in Chinese semi-winter rapeseed

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High-density SNP genotyping arrays are a powerful tool for genome-wide association studies and can give valuable insight into patterns of population structure and linkage disequilibrium (LD). In this study we used the Brassica 60kSNP Illumina consortium array to assess the genetic diversity, population structure and the extent of LD and haplotype blocks in a diverse panel of 203 Chinese semi-winter rapeseed inbred lines. Population structure and PCA analysis using a subset of the SNPs revealed diversification into three subpopulations and one mixed population, reflecting targeted introgressions from external gene pools during breeding. Surprisingly, detailed pairwise LD analysis revealed that average LD at a threshold of r2=0.1decayed around ten times more rapidly in the A genome (250-300 Kbp) than in the C genome (2.0-2.5 Mbp). A total of 1,053 conserved haplotype blocks were detected over a total length of 85 Mbp (7.12% of the genome). The average size of haplotype blocks in the A genome was considerably shorter (63.20 Kbp) than in the C genome (107.35 Kbp). In particular, extremely large conserved haplotype blocks were found on a number of C-genome chromosomes. Comparative sequence analysis revealed conserved blocks containing homologous QTL for flowering time and seed glucosinolate content on chromosomes A1/C1 and A2/C2, respectively. Interestingly, C genome QTL regions showed more conservation of LD than their corresponding A genome homologues. This indicates strong selection for large chromosome regions associated with important seed quality or flowering traits conferred by C-genome QTL. This finding indicates that an increase in genetic diversity and recombination within the C-genome is particularly important to maximise heterosis in Chinese rapeseed hybrids. Genome-wide association studies are also expected to show vastly different levels of resolution across different genome regions.

poster number 5

Identification and characterization of seed longevity genes in barley (Hordeum vulgare)

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Preventing the loss of the genetic variability of cultivated plants and their wild relatives due to changing environmental conditions and cultural practices is a global priority. Worldwide more than six million accessions have been accumulated in ex situ genebanks of which more than 90% are stored as seeds. For crop germplasm preservation seed longevity is of particular importance. Our main goal is to identify and characterize seed longevity genes in barley (Hordeum vulgare).
To allow accurate mapping of quantitative trait loci (QTLs) controlling seed longevity in barley, near isogenic lines (NILs) were derived from a L94 x 116-5 recombinant inbred line (RIL) population. The L94 x 116-5 RIL population was developed from a backcross of the line 116 5 (F5), resulting from a cross between the Ethiopian two-rowed barley landrace L94 and the Argentinean six-rowed landrace Cepada Capa, to L94 (BC1). Backcrossing of one of the resulting RILs, RIL 114 (BC1F12), to L94 (BC2) and subsequent selfing and single seed descent led to the formation of the NILs (BC2F4). A set of 204 polymorphic single nucleotide polymorphism (SNP) markers from Barley Oligonucleotide Pool Assay 1 (BOPA1) and morphological markers (Blp, Vrs1) were used to genotype the RILs (Adimargono et al., unpublished).
Four putative QTLs, identified in the L94 x 116-5 recombinant inbred population (Adimargono et al., unpublished), could be confirmed. RNA Seq analysis of the NILs was employed for mapping of the introgressions and identification of differentially expressed candidate genes and possible downstream targets.
Currently, mapping populations are generated for fine mapping and subsequent identification and cloning of candidate genes. L94 NILs with improved seed quality will be tested employing controlled deterioration tests and ROS related assays in order to identify the processes that are different between these lines.

poster number 6

PlantsProFood – New varieties of narrow-leafed lupin for application in human nutrition

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The seed protein of narrow-leafed lupin (Lupinus angustifolius) is known for its exceptional functional properties as food ingredient and, thus, represents a resource of vegetable protein for human consumption. To take advantage of this potential for a broad range of food applications the regional R&D network 'PlantsProFood' was set up in Northern Germany. Lupins shall be enhanced as a protein resource for food purposes, like ice cream, sausages, bakery products or pasta. The network, consisting of four research institutions and ten local companies, aims at processing the value chain from the development of (I) high-yielding varieties, (II) new extraction and processing approaches towards (III) innovative food.
Requirements for these efforts are high and stable kernel yields of narrow-leafed lupin. This may be accomplished by plant breeding as far as sufficient genetic variability is available. To enlarge the genetic variability of advanced lupin breeding material an EMS (ethyl methanesulfonate) mutagenesis of cv. 'Boruta' was performed and the progeny screened for novel phenotypes.
Phenotypes with a conspicuous novel growth type, such as vigorous growth or high branching compared to the wildtype 'Boruta', were identified and devised to homozygous and stable mutant (M) lines. For some M lines the expected yield potential could be confirmed by significant increase in kernel yields after evaluation under field conditions. For genetic analyses, crosses of the respective M lines with gene bank accessions as well as with the wildtype 'Boruta' were carried out. Segregation analysis of F2 populations suggested a monogenic-recessive inheritance of novel growth types.
Promising M lines are going to be subjected to an analysis of differentially expressed sequences of mutant lines and the wildtype via RNAseq techniques and SNP identification.
Currently, selected SNPs are established via high-resolution melt analysis, genotyped in segregating populations followed by linkage analysis to develop selection tools for marker-assisted selection.

poster number 7

Development of a SNP-assay for selecting drought stress resistance in rye (Secale cereale L.)

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Rye (Secale cereale L.) is known for its outstanding tolerance to biotic and abiotic stresses including drought. Although the application of next generation sequencing recently increased the genomic resources for rye significantly, only limited knowledge is yet available on transcripts involved in drought stress tolerance in this important crop. To provide rye breeders with an easy-to-handle tool for the direct application in breeding, an assay was designed, especially addressing genomic regions involved in the reaction to drought stress. To achieve this, transcription profiling of two elite inbred lines of a German hybrid rye breeding program differing in drought stress resistance was conducted. These genotypes were exposed to drought stress under controlled environmental conditions and showed significantly different reactions upon the stress in terms of biomass production. Plant material from both genotypes was harvested under drought and well-watered conditions to construct cDNA libraries. Conducting a massively parallel sequencing approach of these libraries followed by a de-novo assembly using the “Trinity” tool for data processing, 128,012 contigs were created increasing the sequence information available for rye. This information was extended with the expression level information determined by the “deepSuperSAGE” technology. Combining both information and selecting highly valid single nucleotide polymorphisms (SNP), a total of 4,437 contigs were identified showing significantly differential expression levels between both genotypes and between drought and well-watered conditions. Through the selection process all these contigs are characterized by high-quality SNPs between both genotypes. To further utilize the sequence information, an in-silico mapping was performed using information from the recently published “Rye Zipper”. As a result of this mapping the genomic position of 2,754 contigs out of 4,437 contigs was predicted. All of these contigs were previously filtered for high quality SNPs. Based on the sequence data of the contigs and the positional information from the mapping an assay was designed covering the whole rye genome with SNP markers in approximately equal distances. Based on this information, a SNP assay was developed, that comprises 384 SNPs derived from genomic regions highly differentially expressed under drought stress. To test the applicability of this assay, a bi-parental population characterized for drought stress was analyzed and corresponding SNPs mapped and checked for quality parameters.

poster number 8

Discrimination of alleles and copy numbers at the domestication gene Q using quantitative pyrosequencing

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Speltoid bread wheat spikes can be one reason for increased heterogeneity and consequently rejection of a wheat cultivar candidate. Pyramidal spikes with an elongated rachis and features similar to wild grasses such as tenacious glumes are characteristic for a speltoid bread wheat phenotype. Two copies of the domestication gene Q on the long arm of wheat chromosome 5A show a normal square headed spike morphology. Copy numbers lesser or more than two express speltoid or compact spike shapes, respectively. A quantitative pyrosequencing assay (Förster et al. 2011) was developed to distinguish between normal wheat plants with two copies of the Q allele and aberrants. These aberrants can be aneuploids either with a reduced number of chromosome 5A copies or plants which carry the primitive 5Aq allele. A reproducible determination of the Q gene copy number is now possible, based on homoeologous peak height quantification, from a pyrosequencing-based pyrogram. The calculated Q ratio (5AQ/5Bq and 5Dq) allows to quantify copy number variation at the 5AQ locus.
Based on 10 licensed German wheat varieties, 371 single progeny plants originating from speltoids were molecularly and phenotypically characterized in 2011. Pleiotropic effects of the Q gene on plant height, threshability, spike density and thousand grain weight were observed and confirmed between normal and speltoid offspring. A 98.06% correspondence between spike morphology and the Q gene copy number estimation using pyrosequencing indicates that this method is highly reliable and suitable for high throughput screening. In future, a quantitative pyrosequencing assay may be applied in wheat breeding programs to carry out marker-assisted selection against the presence of speltoid spike aberrants.

References

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poster number 9

Allele mining in wild barley: finding new exotic genes which control flowering time in the barley nested association mapping (NAM) population HEB-25

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Flowering time of crop plants is crucial in practical agriculture, especially for handling the consequences of climate change. Plant breeders have to deal with this problem and take it into account in upcoming breeding programs. A known way of controlling flowering time is the use of genetic resources from wild species. Recently, we have developed the barley nested association mapping (NAM) population HEB-25, consisting of 1,420 BC1S3 lines originating from crosses of the spring barley cultivar Barke with 25 highly divergent exotic barley accessions.The aim of the present project was to find new genes and allelic variants that control flowering time. The identification of quantitative trait loci (QTL) associated with flowering time was performed through a genome wide association study (GWAS). To this end, genotype data were derived from the Infinium 9k iSelect HD chip for barley, which consists of 7,864 SNPs (5,709 are polymorphic in HEB-25) and phenotype data on flowering time were collected in three field trials from 2011 to 2013 at the experimental station in Halle, Germany.The GWAS revealed QTL on all seven chromosomes. Both known regulators of flowering time (i.e. Ppd-H1) and new loci could be identified. This results show that the statistical power to detect QTL in HEB-25 is high. Another advantage of HEB-25 is the presence of heterozygous lines, which is an ideal starting point for high resolution mapping of a candidate gene. Finally, in a further step we plan to embark on cloning and characterizing newly identified flowering genes.

poster number 10

Association analysis of abiotic stresses tolerance in winter faba bean (Vicia faba L.)

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Improving drought and frost tolerance in faba bean (Vicia faba L.) is gaining importance in current day breeding programs. A prerequisite for both marker assisted selection and marker assisted breeding (MAS; MAB) is knowledge and availability of DNA markers in useful association with genes for tolerance to these stresses. Association analysis allows to employ broad genetic material; often uncovering such useful marker-trait associations. For this purpose, $\approx$ 200 pure lines of winter faba beans (representing the main current breeding germplasm) were genotyped using >1000 markers (AFLP and SNP). Drought and frost tolerance aspects were assessed (such as proline and sugar accumulation under drought and symptoms of frost injury) in controlled experiments. High heritability values were achieved ($h2 \geqslant 0.75$) for the traits (both, drought and frost tolerance). Moreover, highly significant correlations (mostly $r>0.80$) were found among stress tolerance subtraits. Several AFLP and SNP markers were significantly ($P<0.00001$) associated with frost and/or drought tolerance. These putative QTL will be further checked and proposed for MAS and MAB to genetically improve winter faba bean. Further findings and a critique of their impact on breeding faba bean will be presented.

poster number 11

Analysis of diploidization rates of Microspores from Intervarietal Substitution Lines of Rapeseed (Brassica napus L.).

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In Brassica species isolated microspore culture protocols are well established and routinely used. Microspore culture for Double Haploid (DH) production is an important technique that produces homozygous lines in one generation thus avoiding several generations of selfing compared to conventional methods. This DH plants can help accelerate crop improvement and breeding programs, produce cultivars, increase plant breeding efficiency, locate genes of importance and be used for constructing genetic maps. For this purposes an efficient DH production is required. There are many reports about agents that can cause high efficient chromosome doubling in isolated microspores of rapeseed but there is also a large variation in diploidization rates between different genotypes after identical treatments. Little is known about the genetic control of these differences. In segregating DH populations, alleles that have an effect on the diploidization rate of microspores are supposed to lead to skewed segregation at markers linked to the loci where the allele are segregating. To localize the genetic factors that control the diploidization rate of microspores in rapeseed a genetic map of 483 AFLP markers was constructed in a population of 197 diploid microspore derived embryos (MDEs) derived from one F1 plant of the cross ‘Express 617’ x ‘RS239’ after colchicine treatment of the microspores. A total of 75 markers in 13 regions showed significant deviation ($P<0.05$) from the expected 1:1 segregation. Five regions were chosen for further studies, which either showed significantly skewed segregation ratios in the diploid MDE population and regular segregations in corresponding BC1 and haploid MDE populations or different patterns of skewed segregation ratios between the three populations in the respective regions. A total of 11 intervarietal substitution lines (ISLs) from the same cross with ‘Express 617’ as recurrent parent with donor segments in these regions were selected. Microspores of the ISLs were isolated, treated with colchicine and cultivated. The ploidy level of the resulting MDEs was determined by flow cytometry to evaluate the diploidization rates of the microspores. Finally, the diploidization rates of the ISLs were compared to the diploidization rate of the recurrent parent of the ISLs, ‘Express 617’.

poster number 12

Development of specific primers for candidate genes involved in frost tolerance of bread wheat (Triticum aestivum)

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Bread wheat (Triticum aestivum L.) is one of the most important crop species worldwide. In many regions of the world, growing of high yielding winter wheat is hampered by insufficient frost tolerance of cultivars available. Therefore, improving frost tolerance by using molecular tools is an important task in wheat breeding. One of the major problems in wheat is the development of locus specific primers for candidate genes which is hampered by the high homology of the three genomes. A method for the development of such primers was developed for five groups of candidate genes, i.e. vernalization response genes (Vrn), tandem duplicated C-repeat binding factors (CBF), 5A cold inducible genes i.e. Triticum aestivum cold-regulated gene Tacr7, defective embryo and meristem genes Dem and calcium-binding EF protein Cab, members of the cold-inducible dehydrin (Dhn) gene family and photoperiod response (Ppd) genes, involved in frost tolerance of cereals.
As initial point for the development of specific PCR-primers for these genes different sequence information, i.e. DNA-, mRNA-, EST- or protein-sequences were obtained from wheat or species related to wheat. In total 33 candidate genes i.e. 11 sequences of Triticum aestivum, 11 of Triticum monococcum, two of Secale cereale and 9 of Hordeum vulgare were employed in different BLAST (Basic Local Alignment Search Tool) searches. The data bank of the International Wheat Genome Sequencing Consortium (IWGSC, http://www.wheatgenome.org/) and the Bristol Wheat Genomics (http://www.cerealsdb.uk.net/)) were used for the identification of homologous genomic sequences. Based on these data a high specificity in primer development was achieved by the re-construction of the exon-intron structure and the placement of primers within the introns or less conserved surrounding regions. All developed primers were tested for functionality / specificity (PCR fragment shows a single band) as well as for the correct chromosomal location by use of the Nulli-tetrasomic (NT) lines. For 33 candidate genes a set of 131 primer pairs was developed using this approach. It turned out that 81 primer pairs corresponding to 23 candidate genes were specific and respective fragments correctly localized to chromosomes, i.e. specific fragments were recorded for 62% of the primers. Out of these a set of 46 fragments corresponding to 23 genes was selected for sequencing and for 91.3% high quality sequences were obtained which will be the basis for association genetics studies based on these candidate genes in order to enhance frost tolerance in wheat.

poster number 13

SIMULATENEOUS GENE EXPRESSION AND SNP ANALYSIS OF THE BYDV RESISTANCE GENE RYD4HB INTROGRESSED FROM HORDEUM BULBOSUM INTO BARLEY

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Barley yellow dwarf virus (BYDV) causes high yield losses worldwide. Thereby aphids play an important role as virus vectors. Yield losses caused by BYDV may be reduced using virus-tolerance genes from the primary genepool of barley. Additionally, complete resistance to the virus has been introgressed from the secondary genepool via interspecific crosses with the tetraploid wild species Hordeum bulbosum (Hb). Previous studies indicate that this resistance was introgressed to barley chromosome 3HL and is governed by a dominant gene, Ryd4Hb. In the present study, a BC2F6 mapping population consisting of 450 individuals was used to establish a molecular-marker map for Ryd4Hb. Massive Analysis of cDNA Ends (MACE) and RNASeq were performed with bulks of homozygous resistant and susceptible genotypes after eliciting the resistance reaction by virus-aphid treatment, to 1) identify genes that were exclusively expressed in the resistant plants and 2) to identify SNPs for marker-assisted selection. More than 200 transcripts were found to be exclusively expressed in the resistant plants and about 400 transcripts with unique sequence (SNPs, alleles) were identified. Including anchor markers as well as markers based on the model genome of Oryza sativa about 45 markers were mapped on chromosome 3HL. Three markers are located in the vicinity of Ryd4Hb and are of potential use for marker-assisted breeding programms.

poster number 14

Dissecting genetic factors in wild barley introgression lines controlling growth under reduced phosphate supply in a hydroponic system.

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Phosphate is a major nutrient required for plant growth, however, phosphate availability is often limited in agriculture. Thus, introducing germplasm and genes, which exhibit an improved phosphate use efficiency (PUE) is important for future plant breeding. We studied genetic factors controlling growth in juvenile plants of wild barley introgression lines (S42IL) under two different levels of phosphate supply in a hydroponic system. In this study, 13 shoot and root traits were measured among 47 S42ILs and Scarlett (control genotype). These traits were tiller number, plant height, root length, shoot dry weight, root dry weight, anthocyanin, carbon content shoot, carbon content root, nitrogen content shoot, nitrogen content root, carbon shoot root ratio, nitrogen shoot root ratio and shoot root ratio length. Our aim was to identify S42ILs and ultimately genes, which show a significant increase or decrease in growth relative to the Scarlett control. The analysis of phenotype data was conducted with procedure MIXED implemented in the SAS software. The post-hoc Dunnett test revealed that 43 S42ILs exhibited at least one significant trait effect compared to Scarlett. In addition, 102 QTLs were detected across both treatments, 88 QTLs within high phosphate and 55 QTLs within low phosphate treatments, respectively. The strongest QTL effects will be verified in independent trials and eventually used for breeding barley under limited phosphate supply.

poster number 15

Mapping QTL for salt tolerance in Brassica napus and Brassica oleracea

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Soil salinity is a major abiotic stress for crop production. Worldwide, about 20% of the cultivated lands are suffering from increasing salinization. Many scenarios were proposed to disentangle this problem; selection for salt tolerant varieties is the most suitable one. Our main objective is to map Quantitative trait loci (QTL) governing the salt tolerance at seedling stage in oilseed rape Brassica napus and Brassica oleracea.

B. napus double haploid (DH) population of 138 DH lines was derived from a cross between the winter oilseed rape cultivar Mansholts Hamburger Raps (++ quality) and the French cultivar Samourai (00 quality). B. oleracea Bo1TBDH population consisting of 138 DH lines was derived from a cross of DH broccoli line "Early Big" and a DH rapid cycling cabbage line "TO1000DH3".

Both populations were tested in pot experiments under growth regime 20 °C at day and 15°C at night. For control 0 mM NaCl was used where salinity treatment was 200mM NaCl for B. napus and 100mM for B. oleracea, respectively. Plant fresh weight (FW), plant dry weight (DW), relative water content (RWC), chlorophyll content (measured with SPAD), and Na+ mg /g DM and K+ mg /g DM content were determined.
In B. napus population several QTL were detected under both control and salt treatment e.g. for FW two QTL were detected, one under control on linkage group (LG) (C03) at 79cM and the second was identified under salt treatment on LG (A2) at 23 cM. There were some hotspots where many QTL for FW, DW, SPAD, and RWC were clustered on LG (C2) and for Na+ mg/g DM and K+ mg/g DM on LG (A9).
Similarly, several QTL were detected under both control and salt treatment in B .oleracea for DW two QTL were detected one QTL under control and one QTL under salt treatment both QTL were localized on LG (C3) at 26cM and 19cM respectively. There were hotspots where QTL for FW, DW, SPAD, and RWC are clustered on linkage group LG (C03) and another group of QTL for Na+ and K+ on LG (C9).
The next step of this research project will be to identify candidate genes which are located close to the identified QTL in B. napus and B. oleracea.

poster number 16

TransBulb: Fine mapping of Rym16Hb by using novel molecular approaches

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Breeding of resistant barley varieties can help to minimize yield losses caused by plant diseases. The wild Hordeum species H. bulbosum makes up the secondary gene pool of barley and is a novel source of disease resistances in barley breeding. Via interspecific crosses of H. vulgare (Hv) and H. bulbosum (Hb) Hb chromatin carrying new resistance genes was introgressed to Hv. One of these resistance genes is Rym16Hb which was introgressed to barley chromosome 2HL and confers resistance to the soil-borne BaMMV/BaYMV-1/-2 virus complex. The present study aims at the use of efficient and innovative molecular methods based on next generation sequencing and SNP genotyping for (I) fine mapping of Rym16Hb, (II) reduction of the introgression size via homoeologous recombination and (III) the development of selection markers for plant breeding. Approximately 80 markers were mapped on the 2HL introgression, designed on Exome Capture data, identified by Genotyping by Sequencing (GBS) or developed on the basis of the orthology of the barley chromosome 2HL and the rice chromosome Os04. Thirty-four recombinants were identified which carry Rym16Hb on Hb introgressions with reduced sizes relative to the original introgression. Closely flanking markers are available for marker assisted barley breeding. Field tests are underway to estimate the influence of individual introgressions on crop yield and the malting quality of winter barley introgression lines is tested. After crossing the resistant parent with current breeding lines, the resistance gene will be genetically fixed in DH lines.

poster number 17

Genetic Fine Mapping of Root Lesion Nematode Resistance QTLs in Barley

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Migratory plant parasitic nematodes from the genus Pratylenchus are major pests in agriculture and attack a wide spectrum of crops leading to heavy losses up to 16% of grain yield in barley. Breeding resistant varieties is the most effective and environmentally friendly approach to control root lesion nematodes.

A doubled haploid population derived from a cross between the Turkish accession Beysehir and the old German variety Valentina were used for genetic mapping of P. neglectus resistance QTLs. A genetic linkage map was constructed using 226 DH lines with 388 AFLP, SSR and CAPS markers that cover 1,051 cM on seven linkage groups. Using nematode numbers which were counted 7 weeks after artificial infection, eight QTLs were mapped by composite interval mapping on six linkage groups (2H, 3H, 4H, 5H, 6H and 7H). Comparative QTL analysis revealed that two major QTLs are located at the same position as previously described Pratylenchus resistance QTLs on chromosomes 5 and 6 (Rlnnp5H and Rlnnp6H) which had been mapped with two different populations (Sharma et al., 2011; Galal et al., submitted). Two markers flanking Rlnnp6H are being used to identify DH lines with recombinations within the QTL regions in a large DH population of Beysehir × Valentina. Currently, 760 DH lines have been genotyped with the two flanking markers and 35 recombinant DHs were identified. Recombinant DH lines will be used for fine mapping of the QTL taking the markers selected by whole genome sequencing of two phenotypic bulks.

Two phenotypic bulks representing the distributional extremes of the mapping population were subjected to whole genome sequencing using Illumina HiSeq 2000 technology. Short reads from the susceptible bulk were aligned to the barley reference genome sequence, and a consensus reference sequence was obtained. Reads from the resistant bulk were mapped to the consensus reference sequence and variants between the two bulks were identified. Homozygous variants densities were calculated across all chromosomes in a sliding window of 1Mb using CLC Genomics Workbench 6.5. Preliminary results show that a unique region with the highest variant density was localized at the same position as one of the resistance QTL. Sequence analysis to identify resistance candidates is in progress.

poster number 18

Radiation hybrid mapping in wheat to resolve the exact position of the Fusarium head blight resistance QTL Qfhs.ifa-5A.

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Fusarium head blight (FHB) is one of the most devastating fungal diseases of wheat in the world. A major resistance QTL of wheat, Qfhs.ifa-5A confers resistance to initial infection (type I) and contributes depending on genetic background up to 25 % of overall resistance. Qhfs.ifa-5A is located in pericentromeric position on the short arm of chromosome 5A. Centromeres are considered recombination cold spot, which makes fine-mapping the QTL position almost impossible.
In order to resolve the position of Qfhs.ifa-5A with a dense marker order, which ultimately should provide a starting point for positional cloning of the QTL, we employed Radiation hybrid (RH) mapping. Radiation hybrid mapping approach does not rely on meiotic recombination between marker loci, but utilizes gamma radiation-induced deletions to map markers onto chromosomes. These deletions are randomly distributed and therefore the mapping resolution should be uniform across the chromosome. We generated two in vivo panels for mapping chromosome 5A, from the spring wheat cultivar CM-82036 and the near isogenic line (NIL) C3, which both harboring Qfhs.ifa-5A. Both lines were gamma-irradiated and artificially crossed with cv. Chinese Spring Nullisomic 5A Tetrasomic 5D to yield the 5A monosomic RH1. 200 RH1 seeds per panel were screened with 10 5AS specific SSR-Marker to assess the overall deletion rates: We found that in average each marker was absent in 6.5% of the lines. 40% of the RH1 carried at least one deletion. Both panels will be compared to another RH-panel of cv. Chinese Spring (CRA - Genomic Research Centre, Fiorenzuola Italy). Ultimately one of the panels will be selected for high-density genotyping to construct a high-resolution map of 5A.
Additionally, a gamma-radiated population of 2000 lines currently in M4, which has been derived from a NIL with the resistant Qfhs.ifa-5A allele in the susceptible background of cv. Remus will be phenotyped for loss of the QTL. These lines allow exact phenotyping for presence or absence of Qfhs.ifa-5A. Susceptible lines will be genotyped for loss of markers in the QTL-region. We will also self-irradiated CM-82036 plants from the RH panel with deletions near the QTL for additional phenotyping and fine mapping.

poster number 19

Pyramiding quantitative resistance genes to increase resistance against powdery mildew (Blumeria graminis f.sp. hordei) in barley.

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Powdery mildew (Blumeria graminis f.sp. hordei, Bgh) is one of the economically most important diseases of cultivated barley. Using resistance genes against Bgh is one way to support a safer and more effective barley cultivation. Plants should have a disease resistance that is not only effective, but also remains durable for a long time. Monogenic resistance, which is comparatively easy to introgress into elite barley cultivars, is often broken down by new pathogen races after a short time. To delay such a fast breakdown of resistance we embarked on an alternative approach. Pyramiding of multiple basal resistance genes into susceptible barley cultivars may consolidate the stability of resistance against Bgh.
For this purpose, we have selected the four candidate genes HvCSLH1, HspGER4, HvCSLD2, MIR6, which are located on independent barley chromosomes. The candidate genes have shown to be effective against powdery mildew resistance in barley or to co-localize with resistance QTLs. They were predicted to play a role in secretory pathways and cell-wall synthesis, respectively (HvCSLH1: Shtaya et al., 2006; HvCSLD2: Aghnoum et al., 2010; MIR6: Johrde, 2010). HspGER4 was previously characterized based on the already described wild barley introgression line S42IL-124 (Schmalenbach et al., 2008).
The four candidate genes are currently stacked into the genetic background of two actual elite barley cultivars, JB Flavour and KWS Bambina. This will result in two pyramiding populations where lines possess between 0 and 8 copies of the selected candidate genes. These lines will be tested in the field and in the green house for their response to Bgh infection as well as to additional barley pathogens.

poster number 20

Identification of bolting related genes of sugar beet by a bulked segregant analysis in combination with whole genome sequencing

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With the advance of next-generation sequencing technologies, the in silico mapping and analyzing strategies have opened an efficient way for rapid discovery of agronomically important genes. The major objective of our research project is to identify bolting related genes in the sugar beet genome through NGS technology. A complete suppression of early bolting has been a major target of beet breeding since the beginning. Besides the major bolting locus BTC1 on Chr. 2, the B2 locus which is not linked to BTC1 has also been suggested to contribute to the annual bolting phenotype of sugar beet. We applied a whole genome resequencing approach to identify the causal mutation for a late bolting phenotype at the B2 locus using a segregating population. An F2 population segregating for bolting and non-bolting plants was produced after crossing a biennial sugar beet (B. vulgaris ssp.vulgaris) mutant and an annual wild beet (B. vulgaris ssp. Maritima). Two pools of homozygous bolting (B2B2) and non-bolting (b2b2) plants were established each consisting of genomic DNA isolated from F2 plants and subjected to Illumina Hiseq2000 sequencing. As a result, a candidate genomic region ~1.5Mb covering the B2 locus on chromosome 9 has been identified by a novel approach. In silico mapping and evaluation of candidate sequences are underway in order to narrow down the candidate region and to characterize the candidate gene.

poster number 21

Barley Flowering: Different FT Homologs with Distinct Functions

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Barley allelic diversity at major flowering genes has contributed to its adabtability to different environments across the globe. Environmental cues, such as temperature and day lenght are preceived by the plant and Genes in the vernalization (VRN) and the photoperiod (PPD) pathways are regulated to adjust the switch to reproductive growth and eventually flowering under favorable conditions. The flowering locus T (FT) like gene (HvFT1) is induced under long day conditions and is the suggested integrator of signals from the vernalization and photoperiod pathways. On the other hand, another FT-like gene HvFT3 is the candidate for the short day response locus (Ppd-H2) that is yet to be fully characterized. We have over-expressed HvFT3, to investigate its role in barley early and late development under different photoperiods. Interactions between the transgene and the VRN and PPD genes were analyzed in different sets of segregating populations. Transgenic plants over-expressing HvFT3 switched earlier to reproductive growth than the wild type plants regardless of vernalization requirement. HvFT3 induced VRN1 but this was not associated with an up-regulation of HvFT1. This could explain why transgenic plants mostly had terminated shoot apical meristem (SAM) development and failed to flower normally. HvFT3 failed to activate the same down-stream target genes of HvFT1 under long day conditions. Our results show that different barley FT homologues had acquired distinct functions which enabled the crop to flower under different photoperiods.

poster number 22

Promising Fusarium Head Blight Resistance in Durum Wheat

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Cultivated tetraploid wheat, especially durum wheat (Triticum durum), is highly susceptible to the wide-spread disease Fusarium head blight (FHB). While many resistance QTL have been reported in hexaploid wheat (Triticum aestivum) the QTL identified to date in tetraploid wheat do not provide satisfactory FHB resistance. Consequently, several groups have introgressed resistance alleles from wild and cultivated relatives into durum. In this study, back-cross lines derived from crosses of T. durum and FHB resistance sources including Triticum dicoccum, Triticum dicoccoides and Triticum aestivum were used as resistant parental lines in several bi- and multi-parental crosses with T. durum cultivars. A large population was developed allowing the evaluation of FHB resistance derived from relatives in an agronomically acceptable durum background. This population was evaluated in 2012 and 2013 in disease nurseries at the site of the Institute for Biotechnology in Plant Production (BOKU, Austria) through artificial spray inoculation of Fusarium culmorum macroconidia at anthesis. The population showed large spectrum of response for FHB resistance ranging from highly resistant to susceptible. FHB severity was significantly negatively correlated with plant height (r=-0.51 in 2012 and r=-0.46 in 2013) but variation for FHB resistance was observed among the short lines (≤ 80 cm height) with several resistant lines. Variation for flowering date was low and FHB resistant lines were present in all maturity groups. A subset of 475 lines will be analysed through both linkage and genome-wide association mapping. The lines will be genotyped in high-density at INRA Clermont-Ferrand (France) using the GENTYANE platform and phenotyped at two locations: Florimond-Desprez in Cappelle-en-Pévèle (France) and BOKU University in Tulln. We expect to unveil QTL linked with resistance and/or increased susceptibility and to evaluate the importance of epistatic interactions for FHB resistance in durum wheat.

poster number 23

Nocturnal Sucrose Metabolism in Barley adapts to diurnal Cycles without Control by the Circadian Clock

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The Circadian Clock is an internal timer system that allows cells to measure time and predict recurrent daily events like dust and dawn. This allows plants to anticipate physiological responses which presumably confers a fitness advantage to the organism1. In Arabidopsis, the Circadian Clock regulates many key parameters of growth and carbon metabolism like photosynthesis and resource partitioning at night.Here we report that nocturnal depletion of the major storage carbohydrate in barley, sucrose, is not controlled by the Circadian Clock. This is in contrast to Arabidopsis, where sucrose supply at night results from degradation of starch reserves, a process tightly controlled by the Circadian Clock. Nevertheless, barley sucrose levels at night decrease in a linear pattern to precisely deplete at the end of 24h cycles.Consequently, sucrose depletion in barley at night is controlled by an unknown but clock-independent mechanism. This offers a new perspective on the control of growth and carbon metabolism in monocots like barley.

poster number 24

HvPHYC is a candidate gene underlying the early maturity 5 locus: interaction with the circadian clock and diversity analysis.

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Photoperiodic flowering is regulated by the interaction between various components of the light perception, transduction and circadian clock pathways. Detailed understanding of gene networks regulating flowering and diversity patterns of their individual components will facilitate exploitation of barley (Hordeum vulgare) genetic resources to boost crop productivity. The so-called Bowman introgression lines carrying the early maturity (eam) mutations, which promote flowering under non-inductive short days, have helped identify and characterize several genes from the photoperiod and circadian clock pathways (e.g. mutations in HvLUX1 underlying eam10 and in HvELF3 – eam8).

In this study, using bulk segregant analysis combined with the high-throughput sequencing followed by the fine-mapping, we identified a mutation causing early flowering of the Bowman(eam5) line. This mutation resides in an extremely conserved region of the barley PHYTOCHROME C gene (HvPHYC) – a homologue of Arabidopsis PHYC. The identical mutation was found in several Japanese barley cultivars. We demonstrated that HvPHYC interacts with the barley circadian clock. In Arabidopsis, PHYC encodes a red/far-red light photoreceptor and its role in the regulation of circadian clock function is not known.

The PHYC locus has participated in adaptation of Arabidopsis and pearl millet genotypes to contrasting climates. To reveal the structure of HvPHYC natural diversity, we re-sequenced a 2045-bp fragment of the first exon comprising conserved domains apparently important for its function from 108 wild and cultivated genotypes. The population genetics analyses suggested that this region of HvPHYC is conserved, under purifying selection and resides within a selective sweep.

poster number 25

The transcriptomes of resistant and susceptible faba bean (V. faba) genotypes during early infection wit the fungal pathogen Ascochyta fabae as revealed by Massive Analysis of cDNA Ends (MACE)

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Ascochyta blight caused by Ascochyta fabae is a major fungal disease of faba bean (Vicia faba L.) with significant impact on yield. Although resistant genotypes are known and a few QTLs for resistance have been described, the molecular mechanisms and genes conferring resistance have not been identified so far. There is a hitherto unmet need for crop improvement by marker-assisted resistance breeding (MAS). In order to detect candidate genes for Ascochyta blight resistance in V. faba for MAS, we studied the early transcriptional responses of tolerant and susceptible faba bean inbred lines to infection with A. fabae. In a first step a collection of resistant and tolerant winter faba bean inbred lines were phenotyped for their reaction to Ascochyta infection. Finally six resistant and six susceptible V. faba genotypes were chosen for a controlled infection experiment at the 4-6-leaf stage. Plants were inoculated by spraying the whole plant with a spore suspension (1x106 spores/ml) until runoff with a hand atomizer. Leaf samples were harvested at 4, 8, 12 and 24h after infection from 2 simultaneous replications of treated and control plants. After positive verification of the expected resistance reaction, 96 total RNAs (2 treatments x 6 genotypes x 4 points of time = 96 individual samples) were isolated, DNase treated and aliquots from leaves 4, 8 and 12 h after infection were pooled for the construction of 24 (2 treatments x 6 genotypes x 2 pools) 3´-specific Massive Analysis of cDNA End (MACE) libraries. Of each cDNA, 1x100 bp were sequenced by means of an Illumina HiSeq2000 sequencing machine. A total of 150 million MACE-tags corresponding to the same number of transcripts were obtained. The resulting MACE tags were quantified and assigned to entries in the public data bases with known function using an established multiple-step bioinformatics including the latest genomic and transcriptomic references. For – potentially faba bean specific - transcripts that could not be assigned to any entry in the data bases, a de novo assembly step is used to establish novel 3'-ESTs. This comprehensive analysis of gene expression will reveal differential responses of resistant and susceptible V. faba lines and will provide novel insight into as yet under-researched tolerance mechanisms of this plant species. Candidate genes involved in tolerance will be verified by qPCR of the original set of 96 individual RNA samples. The current status of the project progress will be reported.

poster number 26

Expression QTL Mapping for Fusarium Head Blight Resistance in Wheat

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To gain insights into the wheat response to Fusarium Head Blight (FHB) and uncover genes linked to known resistance QTL, we performed an expression quantitative trait loci (eQTL) study. eQTL studies allow for mapping of transcript abundances on the genetic map in order to infer genes that are involved in defense against FHB.Our study captured expression profiles of 60k genes (Agilent wheat microarray) at two time points (30 and 50 hours) after inoculation with Fusarium graminearum spores from a population of 200 doubled haploid lines (CM-82036 x Remus). These segregate for the prominent resistance QTL Fhb1 and Qfhs.ifa-5A. We found 400 genes at 30 hai and 5,000 genes at 50 hai differentially expressed (fold change >2, adj.p≤0.05) between the parental lines. We used a part of these to generate transcript derived markers (TDMs), which were used together with SSR and AFLP markers from a previous study (Buerstmayr et.al 2003) to improve the existing genetic map. Reanalysing the phenotypic data confirmed two major QTL (Fhb1, Qfhs.ifa-5A) and identified a novel QTL located on chromosome 6A. eQTL mapping for expression data revealed 14,994 and 13,116 significant eQTL at 30 and 50 hai, respectively. Distribution of these eQTL across the genetic map allowed us to identify eQTL that corresponded to phenotypic QTL (Fhb1, Qfhs.ifa-5A and 6A) and to hotspots. These 8 hotspots (which comprise between 350 to1900 genes) potentially encode for regulatory elements that govern the response to F. graminearum. To gain further insights into the activity of these hotspots and QTL we are currently working on GO enrichment analyses of the co-regulated genes. The results will be presented. Cis and trans-eQTL are defined based on distance of the eQTL from physical position of the respective gene. They are determined by plotting the physical position of eQTL against the genetic position of the TDMs. We mapped 1,500 eQTL, of which the majority (80 %) comprises trans-eQTL at either time points. Few cis-eQTL linked for instance to Fhb1 are interesting candidates for further studies.

poster number 27

Characterization of genomic regions responsible for the outstanding microspore embryogenic potential of the doubled haploid oilseed rape line DH4079 (Brassica napus L.)

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The culture of immature pollen grains, called microspores has much importance in breeding of oilseed rape for the rapid and efficient generation of completely homozygous doubled haploid lines. Despite the progress achieved in optimizing tissue culture protocols, tremendous differences remain among Brassica napus genotypes in their embryogenic response of cultured microspores. The genetic basis of the genotypic differences in microspore culture response is still largely unknown. Among the genotypes known, the Canadian spring cultivar Topas is extensively studied for its excellent microspore embryogenic potential. From this cultivar, the doubled haploid line DH 4079 with an even further improved potential has been developed and is currently used as standard genotype in many investigations related to microspore embryogenesis. This genotype produces many thousand embryos per experiment. In contrast, the inbred line 617 of winter oilseed rape cultivar Express, gives a very low embryo yield in the range of few up to 50 embryos per experiment, under comparable conditions. Furthermore, embryos of DH 4079 and Express 617 show a moderate and a good direct embryo to shoot conversion respectively. The main aims of the present research are: a) to generate a double haploid population of the cross DH4079 x Express617; b) to develop a molecular linkage map of this population; and c) to map QTL for microspore embryogenesis and direct embryo to shoot conversion and to compare positions of QTL with those of candidate genes using available Brassica genome sequences. The development of the DH population was carried out by using in vitro propagated F1-plants of the cross DH4079 x Express617 as microspore donors. A DH population of 207 lines has been generated. Those DH lines are currently characterized with respect to their embryogenic potential and direct embryo to shoot conversion. Results from replicated experiments of the first batch of genotypes will be reported.

poster number 28

Population Structure and Linkage Disequilibrium in German Spring Barley

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Climatic change leads to increased drought and heat stress in Germany. Therefore, plant breeders need to develop new varieties that can maintain yield under stressful environments. Since the advent of high throughput and low cost genotyping assays, the classic forward genetics approach for selecting new cultivars for improved performance by phenotype can be supported by marker assisted selection strategies on a genome-wide scale. In our project we aim to detect genetic associations for agronomic and physiological performance of barley (Hordeum vulgare) under drought and heat stress. A population of 640 spring barley lines of two German breeding companies and a set of 89 landrace lines collected from drought-prone areas of the Middle East and North Africa are field trialled at two locations over two years in Germany. A core collection of 88 lines selected from the breeders and landrace genotypes are analysed for physiological and morphological traits under drought and a combination of heat and drought in controlled growth chamber conditions. All barley lines were genotyped with the Illumina 9k iSelect SNP chip which revealed a total of 4515 biallelic markers with a minor allele frequency of more than 10% and less than 10% missing values per marker. Separate analysis of the two germplasm sets revealed only 3868 polymorphic markers in the breeders set and 5467 polymorphic markers for the landrace set. To examine population structure, Bayesian clustering using the program STRUCTURE was performed and revealed 9 clusters, which separated the exotic from the elite breeding lines. A principle coordinate (PCo) analysis on genetic distances shows two major clusters, one with the breeders lines and one with the landrace lines (PCo 1, 40%, PCo 2, 18%). We detected an average linkage disequilibrium (LD) decay of approximately 5 cM across both germplasm sets, with a higher decay of 21 cM for the breeders population and a lower decay of 1.3 cM for the landrace population. The results show that the occurrence of population structure makes it inevitably to correct for this factor in genotype - trait - association studies and that an average resolution of 5.1 cM can be obtained.

poster number 29

Localization and verification of wild wheat genes controlling 16 traits of agronomic relevance through NAM

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Extending genetic diversity of crop plants is a key instrument to increase biodiversity and sustainability in modern agriculture. One strategy to achieve this is to study and select genetic diversity in populations developed from crosses between elite cultivars and related wild donors. We have developed the spring wheat nested association mapping (NAM) population HEW-2, consisting of 399 BC2S3 lines derived from crosses of the synthetic wild wheat donor accession Syn084L with the spring wheat cultivars Devon and Triso as recurrent parents.
Phenotypic data of the resulting two sub-families (D84 and T84) were collected in field trials conducted in two years, at four locations and under two nitrogen treatments. In total, 16 quantitative traits were investigated, belonging to one of the trait complexes yield, pathogen resistance and baking quality, respectively. In parallel, HEW-2 was initially genotyped with approximately 100 SSRs (simple sequence repeats) and recently dramatically extended with the wheat 90k iSelect SNP (single nucleotide polymorphism) chip. Subsequently, both, phenotype and genotype data were combined to carry out genome-wide association studies (GWAS). For this, a mixed linear model and a multiple regression approach, the latter including the step-wise selection of cofactors, were conducted to identify DNA markers which were associated with the regulation of the respective quantitative trait.
So far, QTLs were identified, which were effective across sub-families as well as restricted to individual families. Likewise, QTLs were identified, which were effective across nitrogen treatments as well as restricted to a single nitrogen treatment. In addition, potentially valuable exotic QTL alleles were located, which improved the trait under study in contrast to the elite genotype. These exotic alleles can be used in wheat breeding to improve the respective trait and to widen the biodiversity of our modern elite wheat gene pool.

poster number 30

Mutations in sinapine biosynthesis genes cause drastic reduction of sinapine in rapeseed (Brassica napus L.)

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Sinapine is the most prominent antinutritive compound in the seeds of Brassica napus L. A reduction in sinapine content will promote the use of rapeseed meal in animal feed and human nutrition. We combined loss-of-function mutations of the two sinapine biosynthesis genes SGT and REF1 and measured quantitative expression and enzyme activities in developing seeds along with sinapoyl ester accumulation in mature seeds in three segregating F2 families. A decrease in the expression of the mutated SGT genes suggested a nonsense-mediated decay mechanism. Significant depletion of SGT enzyme activity in developing seeds proved loss-of-function of both gene copies and ruled out background effects. REF1 enzyme activities showed minor reductions and pointed at different substrate specificities of the paralogs and the presence of unspecific aldehyde dehydrogenases. Sinapine contents in the double mutants dropped dramatically and a maximum reduction of 71 % to a final seed content of 2.4 µg/mg was achieved for a combination of stop codon mutations. The introduction of a REF1 splice site mutation did not result in a decrease in seed sinapine content and expression and enzyme measurements indicate incomplete splicing. Unchanged sinapine contents in single mutants reflect a compensation mechanism, but high reductions in the double mutants rule out a major role of alternative pathways. To our knowledge this is the first achievement of blocking a major metabolite accumulating pathway in rapeseed by EMS mutagenesis. The results cast new light on gene dosage effects and provide valuable genotypes for plant breeders.

poster number 31

Detecting paralog-specific gene expression associated with floral induction in oilseed rape

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Oilseed rape (Brassica napus L.) is a major source of vegetable oil worldwide. Adaptation to different environments and regional climatic conditions in oilseed rape cultivars requires variation in flowering time (FTi) and vernalization. Hence, the identification of genetic factors that promote or inhibit flowering holds an important key for oilseed rape breeding. Brassica napus is a modern hybrid originating from the two diploid species Brassica rapa and Brassica oleracea. Compared to the model plant Arabidopsis thaliana, Brassica napus has a redundant genome potentially containing three to six paralogs per Arabidopsis gene. Hence, understanding FTi regulation in Brassica napus requires studying the complex interplay of highly similar gene paralogs. With the goal of identifying FTi regulators of Brassica napus during floral induction via RNA-Seq, we developed a modular pipeline for the analysis of paired-end reads including multiple tools for read mapping, read counting, and the quantification of paralog-specific gene expression. By comparing young non-vernalized plants versus plants displaying first flowering buds, we found more than 30 paralog-specifically and differentially expressed genes, out of which we validated more than 15 via qRT-qPCR. Our results demonstrate that the incorporation of RT-qPCR profiles for data validation is a crucial step for the RNA-seq data analysis in the allopolyploid B. napus.

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Identification of single-nucleotide polymorphism (SNP) markers and sources of resistance to Xanthomonas campestris pv. campestris in Brassica oleracea L.

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Single-nucleotide polymorphisms (SNPs) and insertion–deletions (INDELs) are currently the important classes of genetic markers for major crop species. In this study, 10 different gene sequences were analyzed to identify SNPs and INDELs in a core collection of 40 accessions of Brassica oleracea L. The results revealed that most SNPs and INDELs were found in non-coding regions. Polymorphism information content values for SNP markers varied. The majority of the identified SNP markers can be readily used for genetic mapping. Furthermore, the results demonstrated the usefulness of existing genomic resources for SNP discovery in Brassica oleracea species.
Sixty-Five accessions of Brassica oleracea species were inoculated with 4 isolates of Xanthomonas campestris pv. Campestris (Xcc). Race type strains of races 1, 3, and 4 were used in this study. The results revealed that plants were more susceptible to races 1 and 4 than to race 3, but this was probably due to a difference in aggressiveness between the strains. Some resistance genes were amplified and sequenced from the accessions studied. Resistance to race 3 was controlled by a single dominant locus. B. oleracea seems to lack useful levels of resistance to the most important races (races 1 and 4) of Xcc. It should be possible to improve this species by incorporating the race-specific resistance genes.

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