ITEMS FROM GERMANY

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Association mapping of agronomic traits exploiting historical field data in winter wheat.

Association-based trait mapping is an innovative methodology in detecting genes and is based on linkage disequilibrium in a collection of unrelated plant material. Studies especially in wheat are rare. We exploited historical field data of a winter wheat collection, using a genome-wide assay with diversity array technology (DArT) markers. In total, 520 polymorphic markers were genetically mapped. Two subpopulations were identified by examining the population structure. The collection was field trailed and phenotyped for agronomic traits in up to eight different years. The associations and the extent of LD in the collection and the two subgroups were calculated with the Tassel 2.1 program using two different models for calculating the associations. The general linear model (GLM) corrects for population structure incorporating the Q-Matrix for the two subgroups while a newer approach is additionally including the Kinship-Matrix in a mixed linear model (MLM), which should further reduce the number of false-positive associations. These two approaches are compared for the trait flowering time. A number of 99 significant marker-trait associations was detected with the GLM, whereas with the MLM, only 14 associations were significant. One association was only significant with the MLM, whereas the others were detected with the GLM as well. The 13 coincident associations are located on chromosomes 1B, 1D, 2B, 2D, 4B, 5B, 5D, 6A, 6B, and 7A.

A genetic linkage map of durum wheat.

Based on a cross between two durum wheat cultivars, 'Omrabi5/Belikh2', a genetic linkage map is being constructed using segregation data from a set of 114 recombinant inbred lines. The parents are known to posses tolerance to several traits associated with drought, heat, and salt stress. Additionally, they carry resistance to main rust races (yellow rust and leaf rust) and *Septoria tritici* but also have good processing quality and yellow pigmentation. The parents were screened with 1,072 GWM, BARC, and WMC SSR markers. A total of 275 polymorphic markers consisting of 161 GWM, 64 BARC, and 50 WMC markers, amplifying 292 loci, were utilized for map construction. Phenotyping for drought- and salt-adaptive traits are being carried out by ICARDA/CIMMYT and will be used to identify and characterize the genomic regions associated with those traits. The linkage map also will prove to be useful for marker-assisted improvement and/or developing tolerant cultivars for drought, heat, and salt stress.

Identification of QTL determining post-anthesis drought tolerance and other agronomic traits in bread wheat.

QTL mapping analysis was applied on a new mapping population (HTRI 11712/HTRI 105), which was developed at IPK, Gatersleben, and contained 133 $F_{2:3}$ families. The genetic linkage map contained 285 SSR loci forming 19 linkage groups. Chromosomes 6D and 4D failed to have proper genetic maps. Population phenotyping on both control and for post-anthesis drought stress was conducted four times, including two greenhouse experiments in 2004 and 2007 and two field experiments in 2004 and 2005. Drought stress was imposed in three experiments using chemical desiccation by spraying with potassium iodide and in one greenhouse experiment by water stress. In both methods, stress was applied from two weeks after anthesis for each $F_{2:3}$ familiy separately. The linear mixed model analysis of variance on

the control and stress condition showed highly significant differences among the $F_{2:3}$ families for the all traits, such as 1,000-kernel weight, seed size, days-to-flowering, number of seeds/spike, weight of seeds/spike, spike length, and plant height, and justified the QTL mapping analysis.

Composite interval mapping analysis revealed 64 and 51 QTL in control and stress condition, respectively. Thirty-seven QTL were repeated either in different experiments or under different stress conditions. The QTL are distributed over all linkage groups except 6A, 6B, and 3D. The number of QTL on the linkage groups were not equal and showed a range from one on chromosomes 1D and 3B to 15 on chromosome 7D. For 1,000-kernel weight, chromosomes 1B and 4B had QTL under control condition, whereas chromosomes 7D and 7A carry the QTL under stress. Both parents contributed increasing alleles for all the traits including thousand-grain weight under stress condition.

Mapping of QTL for terminal heat tolerance in bread wheat.

Post-anthesis high temperature (>30°C) at the time of grain filling is a major cause of yield reduction in wheat in many environments of the world. Hence, finding QTL for heat tolerance is an important objective for future food security.

A QTL analysis of the recombinant inbred population 'NW1014 (tolerant)/HUW468 (susceptible)', segregating for heat tolerance in hexaploid wheat, was completed by applying composite interval mapping. The QTL were detected on chromosomes 2B, 7B, and 7D using three different parameters for heat tolerance: heat susceptibility index of 1,000-kernel weight (HSITGW), heat susceptibility index of grain-filling duration (HSIGFD), and canopy temperature depression (CTD). The QTL for HSITGW were detected on chromosomes 2BL, 7BL, and 7DS in all three environments. The QTL for HSIGFD were detected on the same genomic region of the chromosome 2BL where the QTL for HSITGW was identified. Other co-localized QTL controlling HSITGW and CTD were detected on the long arm of chromosome 7B. One more QTL for HSITGW was detected on the short arm of chromosome 7D.

Microsatellite mapping of a leaf rust resistance gene transferred to bread wheat from Triticum timopheevii subsp. timopheevii.

A leaf rust-resistance gene transferred from the tetraploid wheat *T. timopheevii* subsp. *timopheevii* (genomic composition A^tA^tGG) into common wheat *T. aestivum* subsp. *aestivum* conditioned resistance at the seedling and adult-plant stages in the introgression line 842-2. To determine chromosome location and map the resistance gene, an F_2 population from a cross between line 842-2 and the susceptible wheat cultivar Skala was developed and screened against leaf rust pathotype 77. Microsatellite markers detected introgressions of the *T. timopheevii* subsp. *timopheevii* genome on chromosomes 1A, 2A, 2B, 5B, and 6B of line 842-2. Linkage analysis revealed an association between leaf rust resistance and microsatellite markers located on chromosome 5B. The markers *Xgwm880* and *Xgwm1257* were closely linked to the resistance gene with genetic distances of 7.7 cM and 10.4 cM, respectively. Infection-type tests with three leaf rust isolates resulted in different patterns of infection types of line 842-2 and a Thatcher NIL with the *Lr18* gene on chromosome 5B. The data corroborated the hypothesis of the diversity of the resistance coming from *T. timopheevii* subsp. *timopheevii* The resistance gene of the introgression line 842-2 seems to be different than *Lr18* and, therefore, was designated *LrTt2*.

Leaf rust and powdery mildew resistance derived from Aegilops markgrafii.

A complex crossing program was initiated to detect the number and location of powdery mildew-resistance genes in introgression lines carrying the resistance coming from *Ae. markgrafii* accession S740-69. In addition, the location of a leaf rust-resistance gene originating from the same *Ae. markgrafii* accession and also introgressed in a wheat background should be combined with the powdery mildew resistance mentioned above in one genotype. The results of the segregation analyses at seedling and adult-plant stage were described in 2009 (Ann Wheat Newslet 55:54).

For detailed investigations with microsatellite markers, F_2 generations with resistance to both diseases and for powdery mildew resistance only were selected. The leaf rust-resistance gene in both double-resistant progenies was located on 2AS. The powdery mildew resistance originating from two different introgression lines was identified on chromosomes 7AL for one progeny and 1AS for the other. The same markers that were suitable for the identification of

powdery mildew-resistance genes in the crosses of resistant introgression lines with the susceptible wheat parent Kanzler could also be successfully employed for the identification of the powdery mildew resistance in the double-resistant progenies.

Genetic mapping of ent-kaurenoic acid oxidase genes in bread wheat.

Ent-kaurenoic acid oxidase (KAO) catalysis three steps in the gibberellin biosynthesis pathway, which yields a large hormone family affecting plant growth and development. We performed partial gene cloning and DNA polymorphism-based mapping of three KAO genes in bread wheat. The KAO loci mapped to the distal ends of the chromosome arms 7AS, 4AL, and 7DS, corresponding to the 7BS/4AL translocation region. Co-linearity of the chromosomal regions carrying the KAO genes was shown, suggesting that the KAO genes represent a homoeoloci set. Following the rules of wheat homoeologous gene designation, the KAO genes were designated *Kao-A1* (chromosome 7AS), *Kao-B1* (4AL), and *Kao-D1* (7DS).

Functional allelic diversity at the Rc (red coleoptile) gene in bread wheat.

The wheat *Rc* genes are thought to be regulatory genes in the anthocyanin biosynthesis pathway (ABP), determining specific expression of the ABP structural genes in coleoptiles. The presence of anthocyanin pigmentation in coleoptiles of Russian bread wheat cultivar Saratovskaya 29 (S29) and the standard cytogenetic disomic substitution stock Chinese Spring (Hope 7A) (DS CS–H 7A) is determined by the same gene *Rc-A1* mapped to chromosome 7AS. The *Rc-A1* alleles of S29 and DS CS–H 7A differ from each other phenotypically; S29 has light red coleoptiles, whereas the coleoptiles of DS CS–H 7A are dark red, suggesting that *Rc-A1* may have different transcriptional activity in these two genotypes. The wheat *Rc* genes have not been isolated and sequenced thus far, hindering direct analysis of their expression profiles. However, their activity may be accessed indirectly by analysis of expression patterns of their target genes. Expression of the *F3h-1* gene, encoding one of the key ABP enzymes, flavanone 3-hydroxylase, is activated by the *Rc-1* genes in wheat coleoptiles. In green coleoptiles, *F3h-1* is not active, thus, *F3h-1* is an appropriate target gene that may be used for indirect evaluation of *Rc-1* activity. The patterns of *F3h-1* expression in the coleoptiles of S29 and DS CS–H 7A were compared. There was a significant difference, with *F3h-1* expression being lower in S29 than in DS CS–H 7A. The lower level of *F3h-1* expression in S29 compared to DS CS–H 7A was consistent with the pattern of development of coleoptile pigmentation. This result suggested that there may be functional allelic diversity at *Rc-A1*, which affects the transcription of the *F3h-1* genes in colored coleoptiles.

The effects of growth retardants on 1,000-kernel weight and plant height in wheat.

The effects of growth retardants interfering with gibberellic acid metabolism on plant height have been well documented. We were interested to study additional effects of growth retardants on grain size in wheat lines where plant height and grain size depended on genotype. We investigated the effects of three growth retardants, Regalis, Cylyocel, and Topflor, on the expression of grain size measured as 1,000-kernel weight and plant height in nearly isogenic wheat lines containing the dwarfing gene *Rht12* as well as in wheat lines containing the QTL for grain size *QTgw.ipk-7D*. Plant height was mainly reduced by Regalis and Cyclocel pre-anthesis treatments. A reduction of grain size was caused by a Regalis post-anthesis treatment in most lines, whereas in several cases a pre-anthesis application of growth retardants led to an increase in grain size. In the control blocks, a correlation between grain size and plant height was observed, which remained stable in most treatments except the Regalis pre-anthesis treatment. Our results support the conclusion that gibberellic acids play a role in the expression of grain size and that interference in the GA metabolism can interfere with grain development.

Seed ageing studies in bread wheat.

The influence of long-term storage (natural aging) and artificial ageing on seed germinability were compared using a bread wheat example. Eight lines of differential germinability, after being in storage at low temperature and low humidity for 35 years in the Genebank of IPK, Gatersleben, were used. The seeds were reproduced in 2008, and the renewed seeds were artificially aged by a 72-h treatment with a combination of high temperature and high humidity. The arti-

ficial ageing reduced the germination percentage to a different degree corresponding to the germination percentage of the long-term stored (naturally aged) material. This reduction was significantly less in lines that had maintained high germinability compared to lines with a considerable decline in germinability. The ability of an artificial-ageing treatment applied to fresh seeds to reveal genotypic differences in seed germination capacity comparable to those exposed by long-term natural ageing at low temperature is of importance for seed vigor and seed longevity assessments in both genetic studies on these traits and genebank seed-management activities.

Seed longevity and dormancy in bread wheat.

A QTL-mapping approach was adopted to discern the genomic regions that impart long life and stability to bread wheat seeds. Seeds of the ITMI mapping population were available from regeneration in 2003. Standard germination tests and artificial aging tests were performed. Initial germination percentage ranged from 59% to 97%. Germination percentage after artificial aging ranged from 28% to 90%. QTL mapping revealed one major QTL on chromosome 2A putatively responsible for the higher percentage of germination after an artificial-aging treatment.

The whole population was regenerated in 2009. Seeds were subjected to standard germination tests, artificial ageing tests, and controlled deterioration tests. Initial germination ranged from 24.5% to 98%. Germination percentages after artificial ageing and controlled deterioration ranged from 0.5% to 92% and 0.5% to 90%, respectively. Artificial aging tests revealed two minor QTL on chromosomes 3B and 7A, whereas the controlled deterioration tests identified one major and one minor QTL were detected on chromosomes 1A and 3D, respectively. These lines also were subjected to dormancy tests to discover the relationship between dormancy and longevity. One major QTL for dormancy was discovered on chromosome 4A indicates that there seems to be no relationship between dormancy and longevity for the population under investigation.

Response of the antioxidant glutathione to ageing of wheat seeds.

Seeds can be stored long-term, but their viability is limited. A variety of intrinsic and extrinsic factors influences the longevity of seeds. The viability of seeds in response to ageing correlates with concentrations of the antioxidant glutathione and its half-cell reduction potential.

Viability and changes in the glutathione/glutathione disulphide couple of 120 wheat samples (13 treatments including long-term storage and artificial ageing at 43°C and 18% and 13% seed moisture content) were assessed using germination tests and HPLC analysis, and half-cell reduction potential was calculated using the Nernst Equation.

With a depletion of total glutathione, the total germination of differently treated wheat accessions decreased ($r = 0.73^{**}$). Oxidized glutathione differs between the treatments naturally aged, artificially aged at 13% seed moisture content, and artificially aged at 18%. Half-cell reduction potential also shows a clear difference between the treatments.

Overall, half-cell reduction potential tends to be a good viability marker in wheat seeds ($r = 0.72^{**}$) and, considering the treatments independently, the correlations improve (long term storage: $r = 0.83^{**}$; artificial aging 18%: $r = 0.88^{**}$; and artificial aging 13%: $r = 0.76^{**}$).

Embryo lethality in wheat-rye hybrids.

In crosses between hexaploid wheat and inbred lines of cereal rye, a small number of rye genotypes produce seeds carrying undifferentiated nonviable embryos. Hybrids between such lines and those not giving this phenotype were used as pollen donors in wide crosses with bread wheat to determine the genetic basis of the embryo failure phenomenon. These showed that a single major gene, named *Eml-R1*, is responsible for the embryo lethality character. A set of molecular markers genotyped in an F_2 population between contrasting rye inbreds was used to determine linkage to embryo lethality among a set of F_5 RILs. *Eml-R1* maps to chromosome 6RL in the region of the two co-segregating microsatellite loci *Xgwm1103* and *Xgwm732*.

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ITEMS FROM HUNGARY

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The wheat season. The third extreme drought in one decade characterized the 2008–09 wheat season. A total lack of rain in April and May accompanied by high temperatures caused early maturing and yield decrease. The national wheat average reached only 3.84 t/ha, which was only slightly better than the 3.6 t/ha harvested in the extra dry year 2007. The quality of wheat harvested was good, with low protein in some regions where fertilizer uptake was prevented by drought.

Breeding.

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Breeding. Four winter wheat cultivars were registered in Hungary in 2009.

Mv Menüett (Mv 07-05) is an early maturing cultivar with very good quality, selected from the cross 'F1959W1-2/MV22'. Yield level is slightly higher than that of the existing quality wheats. The cultivar has reliable winterhardiness and good lodging resistance. Dough characteristics are favorable, measured both with Farinograph and Alveograph. The HMW-glutenin composition is 2*, 7*+9, 5+10. Mv Menüett is moderately resistant to powdery mildew and leaf rust and resistant to stem rust.

Mv Karizma (Mv 08-07), an early maturing, facultative wheat with winterhardiness, is similar to the medium frost-tolerant winter wheats, which is sufficient under the average Hungarian conditions. Mv Karizma represents a