ITEMS FROM GERMANY

LEIBNIZ–INSTITUT FÜR PFLANZENGENETIK UND KULTURPFLANZENFORSCHUNG — IPK GATERSLEBEN
Correnstraße 3, 06466 Stadt Seeland, Germany.

A. Börner, A. Bakhsh, E.I. Gordeeva, E.K. Khlestkina, S. Kollers, J. Ling, U. Lohwasser, M. Nagel, S. Navakode, S.V. Osipova, A.V. Permyakov, M.D. Permyakova, T.A. Pshenichnikova, M.A. Rehman Arif, M.S. Röder, M.R. Simon, N. Tikhenko, and Chr. Volkmar.

Whole genome association mapping of resistance to Fusarium head blight and Septoria tritici blotch in European winter wheat.

A total of 358 recent European winter wheat cultivars plus 14 spring wheat cutivars were genotyped (in collaboration with industrial partners) with 732 microsatellite markers resulting in 782 loci of which 620 were placed on the ITMI map. The resulting average marker distance of 7.2 cM allowed genome-wide, association mapping employing a linear mixed model.

A minor allele frequency threshold of 3% (equalling 11 cultivars) was set, and alleles with a lower frequency were excluded from the analysis. After filtering, 3,176 alleles remained and were employed for the association mapping approach. Although no clear population structure was discovered, a kinship matrix was used for stratification.

All cultivars were evaluated for resistance to Fusarium head blight (FHB) caused by *Fusarium graminearum* and *F. culmorum* in four separate environments (2009 and 2010 in Ahlum; 2009 in Cecilienkoog; 2010 in Halle-Bodenwerder; by B. Rodemann, Julius Kühn Institute, Braunschweig (JKI), Germany). The FHB scores based on 'FHB incidence (type-I resistance) / FHB severity (type-II resistance)' indicated a wide phenotypic variation of the cultivars with BLUEs (best linear unbiased estimation) ranging from 0.07 (most resistant) to 33.67 (most susceptible). A total of 794 significant (-log10(p)-value > 3.0) associations between SSR loci and environment-specific FHB scores or BLUE values were detected, which included 323 SSR alleles. For FHB incidence and FHB severity, a total of 861 and 877 individual marker-trait associations (MTA), respectively, were detected. Of these marker alleles, 210, 229, and 194 were significant for BLUE values for FHB incidence, FHB severity, and FHB score, respectively. In most cases, the same marker alleles detected significant associations for all three parameters, i.e., FHB incidence, severity, and FHB score.

Consistent associations detected in three or more environments were found on all chromosomes except chromosome 6B and with the highest number of MTA on chromosome 5B. The dependence of the number of favorable and unfavorable alleles within a cultivar to the respective FHB scores indicated an additive effect of favorable and unfavorable alleles, i.e., genotypes with more favorable or less unfavorable alleles tended to show greater resistance to FHB. Assessment of a marker specific for the dwarfing gene *Rht-D1* resulted in strong effects. The results provide a prerequisite for designing genome wide breeding strategies for FHB resistance.

The same cultivars were evaluated for resistance to *Septoria tritici* blotch caused by the fungal pathogen *Mycosphaerella graminicola* (by B. Rodemann, Julius Kühn Institute, Braunschweig, Germany). The MTA were based on field data in two consecutive years (2009 and 2010 in Cecilienkoog) and genotypic data of 732 microsatellite markers. BLUEs for resistance were calculated across the trials and ranged from 0.67 (most resistant) to 19.63 (most susceptible) with an average value of 4.93. A total of 115 MTAs relating to 68 molecular markers were discovered for the two trials, and BLUEs by using a mixed linear model corrected by a kinship matrix. Two candidate genes, Ppd-D1 for photoperiodism and the dwarfing gene *Rht-D1*, also were significantly associated with resistance to *Septoria tritici* blotch. Several MTA co-located with known resistance genes, i.e., *Stb1*, *Stb3*, *Stb4*, *Stb6*, and *Stb8*, whereas multiple additional MTA were discovered on several chromosomes, such as chromosomes 2A, 2D, 3A, 5B, 7A, and 7D. The results provide proof of concept for the method of genome wide association analysis and indicate the presence of further Stb resistance genes in the European winter wheat pool.

Seed longevity in near-isogenic lines.

The artificial ageing (AA) method was used to compare seed longevity of two Siberian spring cultivars Saratovskaya 29 (S29) and Novosibirskaya 67 (N67), near-isogenic lines (NILs) developed from S29 and N67, and several control cultivars. The AA test was performed at 42°C, 44°C, 46°C, and 48°C in three replicates (100 seeds/replicate). A noticeable effect of AA on germination of S29 and N67 was achieved only at 48°C. We compared germination of seeds of S29 and N67 and their NILs after AA test performed at 48°C. From the two S29-based NILs having purple pericarp color, both showed more than 20% higher germination rate in comparison with their red-grained recurrent parent S29.

From the two N67-based NILs having purple pericarp color, one showed more than 10% higher germination rate comparied with their white-grained recurrent parent N67. The second line did not differ from N67. Microsatellite-based genotyping showed that, unlike other lines, this line is still carrying extended fragment of the donor parent on chromosome 2A, covering both locus for pericarp color and locus corresponding to the QTL for seed longevity described on chromosome 2A earlier. Thus, the donor's allele in the latter locus could negate the positive effect of purple flavonoid substances in the pericarp on seed longevity.

Chromosomal location of genes for tolerance of prolonged drought in bread wheat.

Drought is one of the most damaging environmental stresses affecting grain yield of bread wheat throughout almost the full geographic range of the crop's production. Bread wheat germ plasm includes a measure of variation in drought tolerance, reflected by its cultivation under a wide range of climatic and edaphic conditions. Among its wild relatives are species naturally adapted to droughted environments, and these offer some potential for extending the genetic variability of wheat via wide hybridization and introgression. Variation in tolerance of prolonged drought was identified among a set of single-chromosome, bread wheat substitution lines involving the replacement of each Chinese Spring (CS) chromosome in turn with its homologue from a synthetic hexaploid (*T. turgidum* subsp. *dicoccoides*/*Ae. tauschii*) wheat (SH). Water stress was applied under controlled conditions by limiting the supply of water to 30% from 100% aqueous soil. Our aim was to identify which chromosomes were most critical to the determination of the drought tolerance displayed by CS and SH. The reaction to the resulting long-term drought stress was quantified by three indices, based on grain yield components. The individual chromosome substitution lines varied with respect to their tolerance, particularly those involving either the A or the D genome (in particular, chromosomes 1A, 5A, 1D, 3D, 5D, and 6D). The least tolerant line was 'CS/SH 4B', followed by 'CS/SH 3A', and 'CS/SH 7D'. The best combination of productivity under well-watered conditions and tolerance to drought was exhibited by 'CS/SH 1D'.

Leaf dehydroascorbate reductase and catalase activity is associated with soil drought tolerance in bread wheat. The study was to identify chromosomes that are responsible for the antioxidant enzymes dehydroascorbate reductase (DHAR, EC 1.8.5.1) and catalase (CAT, EC 1.11.1.6) activities in the leaves of wheat plants and stability of yield components under water deficit. The single-chromosome CS substitution lines have separate chromosomes from the donor synthetic hexaploid, an artificial hexaploid combining the genomes of *T. turgidum* subsp. *dicoccoides* (AABB) and *Ae. tauschii* (DD).

The lines carrying a synthetic hexaploid homologous pair of chromosomes 1B, 1D, 2D, 3D, or 4D all expressed a low constitutive level of DHAR and the lines with chromosomes 3B, 1D, 2D, and 3D a low constitutive level of CAT. All were able to increase this level in response to stress caused by water deficit. When challenged by drought stress, these lines tended to be the most effective in retaining the water status of the leaves and preventing the grain yield components from being compromised. As a result of the investigation, we may conclude that DHAR and CAT activities in leaves of wheat at the tillering stage are associated with resistance to drought of the yield components. The discovered genetic variability for enzymes activity in leaves of wheat might be a useful selection criterion for drought tolerance.

Resistance to tan spot and Septoria leaf blotch in hexaploid wheat.

Tan spot and Septoria leaf blotch are very important foliar diseases in wheat and cause significant yield losses. QTL for resistance against both diseases were mapped in different populations. Segregation for resistance among a set of 49 recombinant inbred lines from the cross 'W7984/Opata 85' was used to identify the basis for resistance to tan spot against two fungal isolates. A QTL stable across several environments was located on the short arm of chromosome 6A, linked to the microsatellite locus *Xksuh4c* in seedlings.

To identify the location of the resistance to Septoria leaf blotch, a population of 87 single-chromosome recombinant doubled-haploid lines bred from the cross between the landrace Chinese Spring and a Chinese Spring-based line carrying chromosome 7D from *T. aestivum* subsp. *spelta* was used. Two regions of the chromosome 7D were associated with isolate-specific QTL expressed one at the seedling and another at the adult-plant stage. The seedling resistance locus *QStb.ipk-7D1* was found in the centromeric region of chromosome 7D, which corresponds to the location of the major resistance genes *Stb4* originating from bread wheat cultivar Tadinia, and *Stb5* originating from *Ae. tauschii*. The adult-plant resistance locus *QStb.ipk-7D2* was found on the short arm of chromosome 7D in a similar position to the locus *Lr34/Yr18* known to be effective against multiple pathogens. Composite interval mapping confirmed *QStb.ipk-7D1* and *QStb.ipk-7D2* to be two distinct loci.

Physiological response of wheat-rye hybrids with dwarf 'grass-clump' phenotype to GA3 application.

A gibberellin treatment has been used to try to induce elongation of stems and seed production in the grass-clump wheat hybrids. However, the effect of gibberellic acid on shoot elongation of the wheat–rye hybrid dwarfs from crosses between Chinese Spring wheat (CS) and inbred rye lines V1 and V10, which carry the allele *Hdw-R1b* was never studied before. For the comparative analysis wheat–rye hybrids (CS/V1, CS/V10, and CS/L6), wheat hybrids (Gabo/Sun, Sun/Gabo, CS/Gabo, and CS/Sun), and their parental forms were tested in this experiment.

The effect of gibberellic acid on shoot elongation of the dwarf wheat–rye hybrids, wheat hybrids, and parental forms was different. All parental forms respond with a highly significant increase of the shoot length to GA-application (about 135–190%). Hybrid plants from crosses 'CS/Gabo' and 'CS/Sun' did repond in a similar way, whereas the hybrid plant with dwarf phenotype both in interspecific (CS/V1 and CS/V10) and intraspecific (Gabo/Sun) crosses show no response to GA3 application. We concluded that hybrid dwarfness is not directly connect with gibberrellic acid pathway and has another nature.

Association mapping of aluminium tolerance loci in bread wheat.

Aluminium (Al) toxicity in acid soils remains a constant abiotic stress that affects wheat production in many parts of the world. Al^{+3} ions in small amounts can inhibit root growth thus negatively affecting the water and nutrient uptake by plants. A large body of research has documented the output from bi-parental QTL analyses, which mainly concludes a major effect QTL on chromosome 4DL and few minor loci. Here we report the results from a genome-wide association mapping.

The test material consisted of 96 winter wheat accessions originated from 21 countries and was genotyped with 874 DArT markers. Al tolerance was evaluated by a hematoxylin staining assay. The analysis identified two highly significant loci ($P<0.001$), one on chromosome 1DL and the other on 3BL. The locus on 1DL was assigned to a chromosomal bin, where we identified a relevant Al-tolerance gene candidate *Wali5* (wheat aluminium induced protein) among others. The 3BL locus was assigned to a chromosomal bin, where the possible candidates encoded reactive oxygen species, zinc finger proteins, and metallothioneines, which were reportedly involved in response to Al stress. When the significance threshold was reduced to $P<0.01$, several loci were detected on chromosomes 1A, 1D, 6A, and 7B. Some of the results are in parallel with prior reports and are strong candidates for future research to develop Al-tolerant cultivars suited to grow on highly acidic and Al-toxic soils worldwide.

Identification of QTL determining osmotic stress tolerance and seed parameters in durum wheat.

A mapping population was created from a cross between a drought-tolerant breeding line, Omrabi5, and a salt-tolerant line, Belikh2. A set of 114 recombinant inbred lines (RILs) was examined for their ability to deal with osmotic stress during germination and seedling stage. Seeds of the RILs were subjected to 12% polyethylene glycol (PEG) and compared for vigor and root, shoot, and coleoptile length with the untreated controls. Results were directed to a QTL analysis using 265 microsatellite markers mapped on 14 linkage groups of A and B genomes and spanning 2,864 cM.

Composite interval mapping revealed nine major QTL on chromosomes 1B, 3B, 4B, 6A, and 7B, including five QTL for coleoptile lengths explaining a phenotypic variation up to 28.6%.

Additionally tested seed parameters, such as 1,000-kernel weight, seed area, seed width, and seed length resulted in eight major QTL mainly on chromosome 7B, reaching an explained phenotypic variation up to 57.7%.

Preharvest sprouting and dormancy.

Three wheat populations (ITMI, D-genome introgression lines, and association panel) were studied in order to find responsible loci for dormancy and preharvest sprouting. In addition, two barley populations (OWB and Steptoe/Morex) were investigated. A classical quantitative trait locus analysis was combined with an association mapping approach. Many quantitative trait loci and marker trait associations could be detected on all seven chromosome groups of wheat and on the chromosomes 2H, 3H, 5H, 6H, and 7H of barley. Especially, the known regions on chromosomes 3A and 4A for wheat and 5H for barley were confirmed. Via a candidate homologue search and expressed sequence tag annotation, putative functions could be found. The *viviparous1* gene is located on chromosome 3A, which is associated to preharvest sprouting and dormancy. On chromosome 4A, a protein is detected that belongs to the aquaporin family. Aquaporins are responsible for water flow through the cell membrane. In barley, an association with the aleurain gene on chromosome 5H was found. The expression of aleurain is regulated by abscisic acid and gibberelic acid. From both hormones, an influence on dormancy and preharvest sprouting is known. We concluded that dormancy and preharvest sprouting are very complex traits regulated by multigenes and/or quantitative trait loci.

Susceptibility to wheat midge infestation.

A spring wheat panel consisting of 117 wheat accessions originated from the Gatersleben Genebank collection was cultivated in the field. The population was used for a genome-wide, association mapping analysis for resistance against oange and yellow wheat blossom midges. Midges were surveyed by white water traps. Highly significant marker trait associations (MTAs) were identified on 18 out of 21 chromosomes. For orange wheat midge adults and larvae, 43 and 25 MTA were detected, respectively. Alternatively, 22 and 19 MTA for yellow wheat midge adults and larvae, respectively, were identified. Assessment of ears stored in a freezer for larvae of wheat midges is done at present.

Drought tolerance at early stages in hexaploid wheat.

Two panels consisting of 96 winter and 117 spring wheats were subjected to osmotic stress induced by polyethylene glycol. Root, coleoptile, and shoot lengths and root and shoot dry weights were measured under stress and control conditions. Both panels will be used for a genome-wide, association mapping analysis in order to detect loci responsible for osmotic stress. In addition, the panels will be analyzed for drought tolerance at the adult-plant stage.

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ITEMS FROM HUNGARY

AGRICULTURAL INSTITUTE, CENTRE FOR AGRICULTURAL RESEARCH, HUNGARIAN ACADEMY OF SCIENCES
Brunszvik u. 2, H-2462 Martonvásár, Hungary.
www.agrar.mta.hu

Wheat season. After the extremely dry 2011 (263 mm), the drought continued in 2012. The total yearly precipitation in 2012 reached only 337 mm, compared to the average 550 mm. Due to the dry conditions, no disease epidemics occurred. The national wheat average reached only 3.7 t/ha, which is lower than the last 20-year average. Wheat still performed relatively much better than corn, which had only half the yield compared to the potential. The quality of the harvested wheat was good.