

ITEMS FROM GERMANY

**LEIBNIZ-INSTITUT FÜR PFLANZENGENETIK UND
KULTURPFLANZENFORSCHUNG — IPK GATERSLEBEN
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Whole-genome, association mapping of plant height in winter bread wheat.

The genetic architecture of plant height was investigated in a set of 358 recent European winter wheat cultivars plus 14 spring wheat cultivars based on field data in eight environments. Genotyping of diagnostic markers revealed the *Rht-D1b* mutant allele in 58% of the investigated cultivars, whereas the *Rht-B1b* mutant was only present in 7%. *Rht-D1* was significantly associated with plant height by using a mixed linear model and employing a kinship matrix to correct for population stratification. Further genotyping data included 732 microsatellite markers, resulting in 770 loci, of which 635 markers were placed on the ITMI map plus a set of 7,769 mapped SNP markers genotyped with the 90k iSELECT chip. When Bonferroni correction was applied, a total of 153 significant marker-trait associations (MTAs) were observed for plant height and the SSR markers ($-\log_{10}(\text{P-value}) \geq 4.82$) and 280 ($-\log_{10}(\text{P-value}) \geq 5.89$) for the SNPs. Linear regression between the most effective markers and the BLUES for plant height indicated additive effects for the MTAs of different chromosomal regions.

Analysis of syntenic regions in the rice genome revealed closely linked rice genes related to gibberellic acid (GA) metabolism and perception, i.e., GA20 and GA2 oxidases orthologous to wheat chromosomes 1A, 2A, 3A, 3B, 5B, 5D, and 7B; *ent*-kaurenoic acid oxidase orthologous to wheat chromosome 7A; *ent*-kaurene synthase on wheat chromosome 2B; GA-receptors, such as DELLA genes, orthologous to wheat chromosomes 4B, 4D, and 7A; and genes of the GID family orthologous to chromosomes 2B and 5B. The data indicated that, besides the widely used GA-insensitive dwarfing genes *Rht-B1* and *Rht-D1*, a wide spectrum of loci are available that could be used for modulating plant height in cultivar development.

Morpho-physiological and agronomic indices of plant water status in bread wheat.

We have assessed and mapped QTL for the manifestation of morpho-physiological and agronomic indices of plant water status and related quantitative traits, such as plant height, weight, and dry matter content, in spring wheat. Following the study of 10 agronomic traits, 13 QTL were mapped on linkage groups 1A, 1B, 2B, 2D, 4A, 5A, 5B, 5D, 6A, and 6D. Some of the identified QTL concurrently determined several traits. The physiological components of water status were shown to correlate with quantitative traits in wheat plants, such as plant height, weight, and dry matter content, and the correlation coefficients were calculated for all traits under study. Water retention capacity after 3 h correlated with water retention capacity after 24 h ($r_{xy} = 0.47$). Correlations also were found between water retention capacity after 3 h and plant height at booting stage ($r_{xy} = 0.29$) and between water retention capacity after 3 h and plant dry weight ($r_{xy} = 0.33$). Statistical calculations supported generally observed negative correlation (up to -1) between leaf water and dry matter contents, as well as between the root indices of variance in the mapping population of wheat lines. Obtained results will promote future efforts to fine-map the genes residing within the identified QTL, to eventually clone these genes in order to establish the physiological mechanisms for maintaining water homeostasis in higher plant cells and to accomplish the practical implementation of marker-assisted assessment of water status in wheat plants studied on the basis of morpho-physiological and economical indices.

Studies on salt tolerance in bread wheat.

A bi-parental mapping population (131 recombinant inbred lines) was investigated to study the change in the total root length after salt stress for 5 days. The population was developed by crossing a salt-resistant winter wheat cultivar with a salt-sensitive spring wheat. F₈ plants were used in this investigation. Seeds were germinated on germinating paper (21 x 30 cm) with seeds placed with the embryo down, to allow roots to grow downwards for observing differences in the total length of the roots. The germinating rolls were initially wetted and later placed in water up to a depth of 7 cm for 7 days. Afterwards, a half-strength, Hoagland's solution treatment was applied for the next 5 days. To identify differences in root and shoot length, 200 mM NaCl was added to the solution in comparison to the respective control. Rolls initially were soaked up to a depth of 21 cm for 15 minutes and then kept up to 7 cm from below for the remainder of the period. The coleoptile length of the whole collection in control ranged from 1.8 to 5.3 cm with mean of 3.5 ± 0.84 cm. Root length ranged from 37.1 to 108.3 cm with a mean of 68.08 ± 13.9 cm. Shoot length also showed great variation, where the mean shoot length was 17.0 ± 5.1 cm and ranged from 5 cm to 30 cm. The same population is planted in saline soils. The results obtained from the salt-infected field will be compared with this data to check whether or not the fast screening of wheat germplasm against salt stress is a useful method. The genetic map of this population is under construction. Consequently, the genetic and phenotypic data will be used to map loci for salt stress tolerance in this population.

Studies on frost tolerance in bread wheat using genome-wide, association mapping.

Frost tolerance phenotype scores were collected from several locations in Germany and Russia during two seasons and were combined with the genotypic data in genome-wide association analyses. The genotyping was done employing an ILLUMINA Infinium iSelect 90k wheat chip. The chip carries a total of 81,587 valid and functional SNPs. SNP associations were performed using linear mixed models that evaluated the effects of SNPs with minor allele frequencies >10% individually, adjusting for population structure and kinship. For the population structure analysis, the Q-matrix for three groups was chosen as the best option. Subsequent validation confirmed the results, and using an evolutionary tree calculated by the software PAUP, showed three genotype subgroups: North American, Russian, and North and Middle European. Genome-wide association analyses of the most significant SNP loci identified three and seven positive SNP associations on chromosomes 1B and 5A, respectively, using kinship. Haplotype analysis revealed that most of the significant SNP loci for these positions represent an advantage for the evaluated genotypes.

Induced resistance against Fusarium head blight in bread wheat.

Fusarium head blight (FHB) or scab, mainly caused by *Fusarium graminearum* (Schwabe) and *F. culmorum* (WG Smith) Sacc, is one of the most important fungal diseases affecting wheat in cereal producing areas of the world. The economic losses caused by FHB include yield and quality reduction. The damages induced by the disease are further aggravated by the frequent presence of mycotoxins in affected grains. In the recent years, monoculture, reduced tillage, and maize/wheat rotations have greatly increased the level of inoculum in the soil and, hence, the risk for epidemics of FHB in Argentina. Wheat resistance to FHB is inherited as a quantitative trait governed by polygenes and QTL have been detected on all wheat chromosomes. Because the genetic base of resistance is complex, we selected the most tolerant RILs from the ITMI population to see if their defences can be elicited by applying hormonal inducers or a bacterial suspension. Several of the resistance mechanisms are constitutively expressed or these are elicited by a previous aspersions with hormonal or bacterial treatments. After the treatment, plant defences are 'primed' and respond faster in a more efficient mode when a pathogen attack occurs.

Several RILs, selected by their FHB tolerance, were treated with hormonal inducers of defences and a bacterial suspension (*Pseudomonas* spp.) before inoculation with one aggressive strain of *F. graminearum*. A complete factorial design was performed with untreated plants (controls) inoculated with *Fusarium* (I), pretreated with ethylene (E), treated with E and inoculated (E+I), treated with salicylic acid (SA), sprayed with SA and inoculated (SA+I), sprayed with a bacterial suspension (B), and inoculated after B treatment (B+ I). Elicitation was performed 48 hours before anthesis (Zadoks growth stage 65). Afterwards, each spike was sprayed with 1 mL of the macroconidial suspension using a manual atomizer (constant volume). At harvest, the total number of grains/spike, the number of damaged kernels, the Fusarium index (FI = DK/GS), total weight/spike, and 1,000-kernel weight were calculated.

A part of the tolerant RILs were similar for grains/spike in the complete set of treatments. These lines also had a lower number of damaged kernels and Fusarium index, except when inoculated after SA induction, which showed a slightly higher damage. The TKW was not affected by inoculation in the treated and control plants. On the other hand, inoculated plants of a second group of RILs produced similar GS than control plants, except those sprayed with E, hormone that reduced significantly the grains/spike. The salicylic acid and B treatments induced better performance in this group of RILs, both in the inoculated or noninoculated plants.

Tolerant RILs would have two different types of inducible defense mechanisms. In the first group, the defenses seem to be mediated by ethylene and, in the second group, by the salicylic acid.

Genome-wide, association mapping for resistance components to *Zymoseptoria tritici* in seedlings and its association with plant height and heading date in bread wheat.

Zymoseptoria tritici Desm. (*Mycosphaerella graminicola* (Fuckel) Schrot.) constitutes a major disease problem of wheat, host resistance being the most effective and economic tool to reduce yield losses. Resistance to the disease can be evaluated as percent necrosis and pycnidial coverage. Pycnidial coverage is often more accurate, because necrosis can be masked by senescence and other foliar diseases. An important fact in the search for resistance to this disease is its possible association with plant height and heading date. This work identifies i) marker-trait associations (MTAs) for resistance to *Z. tritici* expressed as necrosis and pycnidial coverage percentage through genome-wide association mapping DArT-based and ii) associations between resistance, plant height, and heading date. Three field experiments were conducted at the Experimental Station J. Hirschhorn, Faculty of Agricultural and Forestry Sciences, National University of La Plata, Argentina, during 2012 and 2013, in a split-plot design. Ninety-six winter wheat accessions from 21 countries were inoculated with two isolates from two locations from Argentina (Pla and Nueve de Julio) at the 3-leaf stage in both years. For two of the experiments, severity (expressed as necrosis percentage and pycnidial coverage percentage) in the first three leaves was scored, and heading date and plant height were evaluated for the three experiments.

A phenotype–genotype association analysis, employing the general linear model and the mixed linear model, was performed with the Tassel 2.1 software. Only MTAs significant with both models were considered. For necrosis percentage, three MTAs for *Z. tritici* resistance were detected on chromosomes 1A (two) and 6B with the isolate from Pla, whereas four significant MTAs on chromosomes 1B (two), 2A, and 2D were effective against the isolate from Nueve de Julio in the two experiments analyzed. Regarding pycnidial coverage percentage, six significant MTAs for *Z. tritici* resistance were detected on chromosomes 1A (two), 1B (two), 5B, and 7A with the Pla isolate, and five significant MTAs on chromosomes 1A (two), 1B (two), and 7D with the Nueve de Julio isolate, in the two experiments analyzed. On the other hand, five significant MTAs for heading date were detected on chromosomes 1B, 2B, 4B, 5D, and 6A, and four significant MTAs for plant height were identified on chromosomes 2B, 3A, 4A, and 7A, for the three experiments analyzed. Some of the MTAs for *Z. tritici* resistance were isolate specific whereas others were common to both isolates.

Additionally, a correlation analysis was performed in which both necrosis percent and pycnidial coverage percent were negatively associated with heading date ($r = -0.18$ and $r = -0.23$ for the Pla isolate and $r = -0.41$ and $r = -0.36$ for the Nueve de Julio isolate), whereas no significant association with plant height was found; except for percent necrosis for the Nueve de Julio isolate ($r = -0.25$).

Association mapping for resistance against insect pests in bread wheat.

A spring wheat panel consisting of 111 different genotypes from 27 countries and a winter wheat panel of 96 accessions from 21 countries were cultivated in the field on different places from 2011 to 2014. The population was used for a genome-wide association mapping analysis for resistance against different insect pests (aphids, *Oscinella frit*, orange and yellow wheat midges (*Sitodiplosis mosellana* (Géhin)), *Contarinia tritici* (Kirby), and the saddle gall midge (*Haplodiplosis marginata*)). The data was gathered using pheromone traps, white water traps, and evaluating wheats.

After sampling all traits, we calculated an association study to find characteristic MTAs for resistance. For resistance to *Ocinella frit*, we detected 41 MTAs in 2013, 44 MTAs on the different wheat chromosomes for the aphids, and nine MTAs for the saddle gall midge in 2012–13. The orange and yellow wheat midges were surveyed by white water traps. Highly significant, marker-trait associations were identified in 2013 on 18 out of 21 chromosomes. For orange

wheat midges adults and larvae, 43 and 25 MTAs, respectively, were detected. Alternatively, 22 and 19 MTAs for yellow wheat midge adult and larvae, respectively, were identified. The panels show wide differences between the individual insect pests.

Genetic diversity in old bread wheats from Bulgaria.

A collection of 60 historic wheat cultivars released in Bulgaria from the beginning of last century to the early 1970s as products of traditional farmers' selection and early breeding activities was assembled. This old germplasm had evolved from a broader gene pool and, therefore, is a valuable though yet underutilized resource for breeding purposes. Genetic diversity was studied in different sets of genotypes with regard to the phenotypic characteristics, growth habit, plant height, earliness, agronomic traits, disease resistance, nitrogen (N) use efficiency, and molecular variability. Among this germplasm, potential sources of good productive potential and lodging resistance were identified. One-third of the accessions demonstrated high to moderate resistance to yellow rust (*Puccinia striiformis*) in field conditions during the epiphytotic development of yellow rust throughout Europe in the spring of 2014. Depending on the environment, N use efficiency in 21 accessions at two N fertilizer levels (N_0 and N_{12}) and two environments differing by soil characteristics and crop predecessor showed variation with respect to the efficiency and N responsiveness. Under more extensive conditions with poorer soil characteristics and a maize predecessor, the majority of old genotypes were N efficient, whereas on haplic chernozem and a pea predecessor, the vast part of old germplasm was N inefficient. The study of genetic variation and distinctiveness among 28 accessions as revealed by microsatellites showed high allelic richness (173 alleles at 25 loci on 14 chromosomes, 6.9 average number of alleles/locus), 0.68 average PIC, 49 unique alleles at 18 loci, and 30.9 % average cultivar heterogeneity.

Genetic diversity in Iranian bread wheat.

A study on 500 bread wheat accessions was conducted to determine genetic diversity based on morphological traits and ISSR makers. The accessions were provided kindly from the IPK–Gatersleben genebank and originated from Iran, Afghanistan, India, Turkey, Pakistan, Iraq, Nepal, Tajikistan, and some other countries. In order to evaluate genetic diversity based on morphological traits, pure lines, derived from the bread wheat accessions, were cultivated and evaluated in a field experiment based on an augmented design with three cultivars as control in 2013. Traits such as day-to-flowering, spike length, plant height, number of grains/spike, 1,000-kernel weight, grain length, grain width, flag leaf width, flag leaf length, peduncle length, and awn length were investigated. Results from these descriptive statistics showed that the maximum coefficient of variation of the phenotypic traits belonged to the awn length and the lowest for the days-to-flowering. A simple correlation analysis showed the highest correlation between the grain weight and grain width. A cluster analysis based on the morphological traits divided the genotypes into four groups.

Ten ISSR markers were used to study genetic diversity on the genome level among the genotypes. This part of study still is in progress and, after that, the results from each of the two parts can be compared. The above-mentioned genotypes are under investigation for salt stress based on hydroponic culture.

Seed longevity in bread wheat – artificial ageing vs. long-term storage.

Recombinant inbred lines (RILs) of the ITMI mapping population were analyzed after artificial ageing (treatment 1 was 100% RH, $43\pm 0.5^\circ\text{C}$, 72h; and treatment 2 was 18% seed moisture content, $43\pm 0.5^\circ\text{C}$, 72h) as well as after long-term storage ($10^\circ\text{C}/50\%$ RH) for up to 14 years. The RILs were reproduced either at experimental fields at IPK in Gatersleben, Germany, or at the University of California Intermountain Research and Extension Center in Tulalake, USA. After ageing, two to four reps of 50 seeds were subjected to standard germination tests and data obtained were used for QTL analysis.

QTL detected after artificial ageing were found on chromosomes 1A, 1D, 2A, 2D, 3B, 3D, and 6B. In contrast, the loci detected after long-term storage were detected on chromosomes 4B, 5A, and 5B. Not only the different growing seasons, but also the different storage and ageing conditions, yielded different loci. The many different genomic regions contributing to the genetic determination of seed longevity underline the complexity and polygenic nature of this trait.

Contradictory mapping positions confirmed that growing and/or storage/ageing conditions exert a large influence on seed longevity.

The use of wheat–barley addition lines to verify a candidate gene for a regulator of anthocyanin biosynthesis in the leaf sheath.

Anthocyanins are implicated in plant resistance to a number of abiotic and biotic stress factors. Anthocyanin pigmentation of leaf sheath is determined by the *Pls-1* genes in wheat (chromosomes 7A, 7B, and 7D) and the *Ant1* gene in barley (chromosome 7HS). The complete set of wheat–barley addition lines (*T. aestivum* subsp. *aestivum* cv. Chinese Spring + *Hordeum vulgare* cv. Betzes) was used to localize the *HvMpc1* gene, a candidate gene for *Ant1*. The *HvMpc1* gene is a homolog of the maize gene *C1* encoding R2R3 MYB factor regulating anthocyanin biosynthesis. R2R3 MYB is a large family of transcription factors encoded by genes distributed on all chromosomes and, therefore, primary verification of the selected candidate genes should be the testing of their chromosome location. The sequence of *HvMpc1* was amplified using a specific primer pair in wheat–barley addition lines carrying chromosome 7H or its short arm, suggesting *HvMpc1* to be a proper candidate gene for *Ant1*. The *HvMpc1* sequence can be useful for further isolation of candidate genes for wheat *Pls-1*.

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ITEMS FROM HUNGARY

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The Martonvásár Cereal Genebank.

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The main task of the Martonvásár Cereal Genebank is to collect, preserve, and maintain wheat species and genetic reserves from related species, and to make detailed investigation on the quality, agronomic value, and biotic and abiotic resistance of the accessions.

The Martonvásár Cereal Genebank is divided into three main parts: a breeder collection, the genetic stock collection, and a set of wheat wild relatives.

Breeder collection. The largest of the collections, the breeder collection consists of more than 11,500 accessions of breeding stocks, cultivars, and landraces. A majority of the accessions are *Triticum aestivum* (90%)