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Examination of genetic components determining the environmental adaptation of bread wheat (*Triticum aestivum* L.)

Main points of the PhD thesis

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1 BACKGROUND AND AIMS

By right of its part in sustenance bread wheat is one of the most important objects of genetic examinations among agricultural plants in Hungary as well as the world. Due to its extraordinary genetic diversity this species has a wide environmental spreading. One of the most important components of the regional adaptation are plant development and flowering time, which are determined to a great extent by gene groups that regulate vernalization requirement, i.e. the cold period that induces the transition from the vegetative to the generative phase (*VRN*), photoperiod sensitivity (*PPD*), earliness per se (*EPS*) and height-reducing genes (*RHT*). This complex process is fundamentally influenced by environmental factors such as annual changes in photoperiod, high and low temperature periods, or intensity and spectral composition of light, which directly affect the plant developmental phases. The main components of this complex regulatory process have been revealed both in wheat and barley, but the molecular-genetic processes of their adaptation to the wide range of environmental conditions are not yet fully understood. Under field conditions, the various environmental factor combinations experienced in different years result in considerable variability in the phenotypic effects of the individual alleles of these genes, often leading to contradictory findings. The analysis based on molecular markers enables to eliminate the modifying effects of the environment. With help of markers genetic maps can be prepared and QTLs (Quantitative Trait Locus) relating to particular features can be determined. The LD-based (Linkage Disequilibrium) association analysis (GWAS: Genome-Wide Association Study) is an effective method to determine the connection of natural genetic variability with phenotypic traits in a larger genotype collection. The aim of our study is to investigate the flowering time of a wide range of wheat varieties using comprehensive phenological and molecular-genetics methods. This was planned to be achieved with the elaboration of the following tasks:

- Characterization of major alleles of the two most important development regulator gene families (*VRN1* and *PPD1*) with specific molecular markers connected to the known functional site polymorphisms in these genes in a collection of 683 wheat genotypes. As a results of this we are able to determine the territorial distribution of various allele groups and the possible correlations between the alleles and the time required for heading. Based on these informations an association panel with a wide range of genetic diversity

will be evolved for a more comprehensive analysis between plant developmental phases and yielding ability.

- Characterization of genetic diversity of the association panel using high-efficiency marker technology. The diversity examinations will be extented for determining the population structure as well as the frequency of gene alleles, with key genetic roles in plant development phases and plant growth habits.
- Composition of LD-marker map of the association panel. These markers are originated from different high-efficiency marker technologies as well as from gene specific markers.
- Comprehensive examination of developmental patterns and yield components of genotypes belonging to the association panel and the correlation of these two trait groups evaluated in a series of field experiments combining different years and sowing times.
- Determination of correlations between the phenotypic and molecular genetic data matrices using genome wide association analysis.

2 MATERIALS AND METHODS

2.1 Plant materials

All plant samples were originated from the winter wheat gene bank of MTA-ATK and were chosen on the basis of breeding location and flowering data recorded in previous experiments. The geographical distribution of the investigated genotypes is the following: 521 from Europe, 62 from Asia, 6 from Africa, 90 from America and 4 from Australia. Both old and new breeding materials were involved in the experiments; some were used to be grown widely, some are important in current wheat production systems. The aim was to use a heterogeneous gene pool in the experiments that contains all the main allele types of the genes in question. From the initial large genotype collection 94 photoperiod-insensitive and 94 photoperiod-sensitive genotypes were chosen based on the two main allele types of the *PPD-D1* gene. These 188 genotypes (MTA-ATK winter wheat GWA panel) were included then into more comprehensive phenotyping and genotyping experiments.

2.2 The field experiments

The field phenological investigations of the genotypes were conducted in the following years in Martonvásár:

1. 2010/11 (sowing was in the beginning of October) [*including of 683 wheat genotypes*]
2. 2011/12 (sowing was in the beginning of October) [*including of 683 wheat genotypes*]
3. 2012/13 (normal autumn sowing: beginning of October; late autumn sowing: end of November) [*including of 188 wheat genotypes*]
4. 2013/14 (normal autumn sowing: beginning of October; late autumn sowing: middle of November) [*including of 188 wheat genotypes*]
5. 2014/15 (normal autumn sowing: beginning of October; late autumn sowing: beginning of November) [*including of 188 wheat genotypes*]

The experimental design of the GWA panel was the same in every year and sowing period. 188 genotypes were sown without replications, while two (‘Mv Toborzó’ and ‘Mv Verbunkos’) were sown 7- 7 times evenly spaced across the experimental field as controls. The experimental plots measured 0,4 × 2 m, two rows were sown for each genotypes with a distance of 20 cm.

2.3 Phenotypic descriptions

Four healthy, near-uniform plants were chosen from each plot for regular scoring of plant height (twice a week) and three development phases, which are the followings on the Zadoks-scale: Z31 (first node appearance at the base of the main stem), Z49 (spike located in the upper part of the flag-leaf sheath) és Z59 (spike fully emerged from the flag-leaf sheath). All three development phases were characterised with the number of days from 1 Jan. of the given year. For the genotypes (188), the effective thermal time was also calculated in addition to the calendar time. The effective thermal time is the sum of the daily thermal time modified with the day length value and the average vernalization requirement of the plants. SPTV = TT × FV × FP, where TT: the daily accumulated thermal time; FV: the vernalization factor; FP: the photoperiod factor. We have developed an objective phenotyping method which is allowed to match the regression equations between the regular plant height measurement data and the time points. This made it possible to determine the start, the end, the interval and the rate of the intensive stem elongation phase (Z30, ZSE, LSE and SG). The length of the last internode, average plant height and length of the main spike were determined at the end of the physiological maturity phase. The plants were grown to full maturity and several yield components like grain number, grain weight and thousand-kernel weight were also scored.

2.4 Genetic characterization of wheat genotypes

For genetic characterisation we used gene-specific markers connected to *VRN1*, *VRN2*, *VRN3*, *PPD1* and *EPS* genes, while in the case of *VRN-A1* and *PPD-B1* genes the determination of copy number variation were applied. The copy number of these genes were estimated relative to the reference gene *TaCO2* using a multiplex TaqMan® assay in cooperation with IDna Genetics Ltd. (Norwich Research Park, Norwich, United Kingdom). The DNA samples of the 188 genotypes were also characterized with high-efficiency SNPs detecting marker technologies. The DArT and the 15K Infinium analyses were achieved by the Diversity Arrays Technology (Triticarte-Pty Ltd. CSIRO, Yarralumla, Australia) and the TraitGenetics GmbH (Gatersleben, Germany). The KASP-marker analysis was performed in John Innes Centre (Norwich Research Park, Norwich, United Kingdom) according to the manufacturer's instructions. With the KASP-marker system we have characterized those chromosome regions of the 188 wheat genotypes which were only partially or not covered with DArT markers. In order to detect additional polyporphisms we have involved 103 KASP markers in this examination. With 14 gene specific KASP-

markers we have also focused on those genes (*VRN*, *PPD*, *PHY*, *FT*, *RHT*), which affects significantly the heading time in cereals.

2.5 Data analysis

The affinity matrix and dendrograms were carried out using UPGMA (un-weighted pairgroup method using the arithmetic mean) grouping of TASSEL 3.0 software package. The population structure was determined by the Structure program. GWAS analysis was performed by the GenStat® (version number 18) statistical program. The Eigen-matrix values of the examined varieties showing significant level in the principal component analysis (PCA) were used to eliminate fals positive values. The threshold values were determined by Q-Q plots making it possible to designate the significant Marker-Trait Associations (MTA). The relationships between marker loci and phenotypic traits were determined by multiple regression analysis (R^2 value). The variance analysis of phenotypic data was determined by Linear Mixed Model (LMM). PCAs were used to determine the interactions and relationships between the developmental phases and each allele types. General Linear Model (GLM) and multiple regression analysis were applied to determine the possible effects between the developmental parameters and each genes using the Statistica 6 software package.

3 RESULTS AND DISCUSSION

3.1 Genetic diversity examinations with molecular marker systems

One of our aims was to characterize the wheat gene bank of MTA-ATK with molecular markers, including agronomically important genes as well, and to analyse the genetic diversity of a winter wheat association panel. As plant development basically determines ecological adaptation and has a significant effect on the yielding ability, thus we focused on the developmental genes in the case of the gene bank samples.

3.1.1 Characterization of the winter wheat genebank of MTA-ATK

As a first step, 683 genotypes were characterized with gene specific molecular markers determining the major alleles of genes responsible for vernalization response (*VRN-A1*, *VRN-B1* and *VRN-D1*) and photoperiod sensitivity (*PPD-B1* and *PPD-D1*). We have confirmed, that the majority of the genotypes exhibited winter seasonal growth habit, with only limited numbers showing facultative growth habits. Accordingly, analyses with gene-specific molecular markers confirmed the overwhelming presence of winter alleles in the three *VRN1* genes, while the various dominant alleles of the *VRN1* genes were only characteristic to 6-7% of the samples. This ratio is in good agreement with results published for winter wheat genotype collection. The ratio of the photoperiod-insensitive allele of the *PPD-D1* gene was 57% among the samples. This allele type was more frequent in the eastern, southern and south-eastern regions of Europe, while in Western Europe the photoperiod-sensitive allele has been found to be more common. In Central Europe the photoperiod-insensitive and sensitive alleles of the *PPD-D1* gene occured at similar frequencies. In the case of the *PPD-B1* gene, there is much less information. In our study, we proved that the frequency of the photoperiod-insensitive allele was relatively high even in a mostly winter wheat germplasm. This allele was detected in 22% of our genotype collection, in breeding materials from Asia and America, in decreasing order of frequencies. The incidence of the photoperiod-insensitive allele of the *PPD-B1* gene in Europe was noted almost exclusively for genotypes from the central and south-eastern regions. While in the cases of the *PPD-D1* and *PPD-A1* genes, the genetic bases of the insensitive allele could be associated with a larger deletion in the promoter region, resulting in one distinct insensitivity allele at both genes, for *PPD-B1* increase in gene copy number results in elevated gene expression

and, consequently, in photoperiod insensitivity. This region proved to be quite variable both in copy numbers and the intercopy structure type between the various copies, probably due to unequal crossing over. In this winter wheat collection, the ratios of two and three copy versions of the insensitive allele were similar (around 1/3 of all the insensitive genotypes, respectively), while the ratio of the four-copy version was lower and characteristic of about 25% of the insensitive genotypes. The vast majority of the four-copy *PPD-B1* genotypes were of European origin. In this winter wheat sample, however, not only the copy number variation was characterized, but also the presence of the truncated gene and the intercopy structure type. From the aspect of intercopy structure, more than 50% of the *PPD-B1* insensitive alleles were of 'Chinese Spring' type, while the frequencies of the 'Récital' (usually to be found among the European varieties) and 'Sonora' types (usually characteristic to the American varieties) were similar to each other (26.0 vs. 23.3%, respectively). As a result of the copy number and intercopy type variations, nine various versions of the insensitive allele were identified, in addition to the two genotypes with null alleles. The low ratio of the null allele type is in good agreement with results experienced by other authors. We proved that the allele phases in *PPD-D1*, *PPD-B1* and *VRN-D1* played significant roles in determining heading under field conditions. The significant phenotypic effects of *PPD-D1* on heading are well documented in different genetic backgrounds. Under the environmental conditions around Martonvásár, Hungary, *PPD-D1* also had the largest genotypic effect on plant development under field-grown conditions, and the presence of the insensitive allele resulted in accelerated plant development in both years. Here we report among the firsts the significant associations between the *PPD-B1* allele type and heading in field experiments. We found that the second largest phenotypic effect was due to the allele types in the *PPD-B1* locus which was connected not only to the copy number but also to the intercopy structure type and to their interactions. In field-grown conditions the intercopy structure type had a stronger effect on heading than the copy number. The average values of wheat genotypes with the 'Récital' intercopy type-insensitive allele was the latest to head, while the 'Sonora' type was the earliest in both years. The copy number variation also resulted in altered phenotypic reaction. In general, the presence of three copies resulted in the earliest heading, followed by the four-copy type; the two-copy type was the latest to head. Our results, however, indicated that the effect of copy number was strongly dependent on the intercopy type, and the better understanding of this phenomenon requires further systematic

research. Under Central European conditions, the vernalization requirement of the winter wheat genotypes is completely saturated during the winter. Although the allele types of *VRN-A1*, and to a lesser extent *VRN-B1*, play an important role in the determination of the vernalization requirement, the results obtained in our study indicate that they had no significant effects on the phenophases of Z49 and Z59, probably due to the saturated vernalization requirement. The spring (dominant) allele of *VRN-D1*, however had a significant associations with the *PPD-D1* and *PPD-B1* genes. The difference between the average values of the photoperiod-insensitive and sensitive allele types of both *PPD1* genes was four days, in the presence of the winter allele type of *VRN-D1* gene, while this value increased to seven days in the presence of the spring allele type, underlining the epistatic interaction between these genes. The inclusion of *PPD-B1* copy number and a more precise resolution of the sensitive alleles of *PPD-D1*, together with the *VRN1* series, lead to the gene model explaining 53% of the phenotypic variance. Based on the main allele types of the plant developmental genes, 12 main allele groups were observed. The earliest genotypes carried the photoperiod-insensitive alleles in both the *PPD-D1* and *PPD-B1* genes, the dominant (spring) allele of the *VRN-D1* gene and the recessive (winter) allele of the *VRN-A1* and *VRN-B1* genes. The late-heading genotypes were found to carry the sensitivity allele for both photoperiod sensitivity genes, irrespective of the allele combinations in the *VRN1* genes.

3.1.2 Genetic characterization of the association panel of MTA-ATK winter wheat

The effect of *PPD-D1* gene on plant development proved to be the strongest under field conditions. Due to this fact we have chosen 94 genotypes from the photoperiod insensitive and 94 from the sensitive allele types. This panel, including 188 genotypes was characterized with several high-efficiency marker techhnologies. Our aim were to determine accurately the genetic diversity of the panel and to compose the LD-based marker map. All these are essential components in the analyses of whole genome association mapping. To ensure genetic diversity within a crop species is fundamental in any successful breeding programme; this can contribute to preserve a sufficient pool of favourable trait sources enabling the selection of better adapted breeding materials. The rate of genetic diversity among breeding varieties is a very important information for breeders. The analysis of the population structure and genetic diversity of the association panel was based on the DArT markers. The

683 wheat genotypes could be devided into four groups which showed significant correlation with the geographic origin. Thus in general, the first group contains American and Asian varieties, the second one involves Central European, the third includes Hungarian and South European and the fourth contains breeding materials from the western region of Europe. The coincidence of the population structure with gene alleles can seriously bias the association analyses, but this can be eliminated by using the relationship matrix of the varieties for modifying the probability thresholds. The varieties of central and south-eastern regions of Europe can be found in all of the four groups. Our results are in agreement with other authors' statements; the genotypes of East-European origin proved to have wider genetic diversity, than the varieties of West and North Europe. This phenomenon can be explained with the differences in the environmental conditions, soil capability and also breeding practices. The isolation line determined by the Alps and the Carpathian can be also the cause of the differences between western and south-eastern areas. The photoperiod-sensitive alleles type of *PPD-B1* and *PPD-D1* genes were detected to the largest extent among the samples of the fourth group (86 vs. 75%, respectively). At the same time in the first and third group we have detected higher proportion of the photoperiod-insensitive allele of the two *PPD1* genes (30 vs. 43%, and 57 vs. 87%, respectively). 12368 polymorphic markers originated from the 15K SNP Infinium examination were the basis of the LD-map used in the association analysis. After eliminating rare alleles and markers showing complete linkage and including the DArT, KASP and gene specific markers, the final sum of the markers in the LD map was 7851. Our LD map is characterized by the alterations of densely and poorly marker-covered chromosomal regions. This phenomenon can be traced back to the characterisitcs of the wheat genome, its self-pollinating nature and that most of the wheat varieties were bred after the Green Revolution (between 1970 and 2005). As the marker distances of the LD-map originated from meta-analyses of several bi-parental marker linkage maps, it can be assumed, that the majority of the wheat varieties are the same in the poorly covered chromosomal region, thus not limiting significantly the whole genome association analyses.

3.2 Effects of years and sowing time on plant developmental phases and yield components of the association winter wheat panel

The GWA panel was involved in field experiments, in which we examined in detail how sowing time and years infuence plant developmental phases and yield

components. We also analysed correlations between trait groups. A detailed physiological and genetic knowledge on starting date and lenght of the plant development phases of wheat or discovery of the correlation with yield components enables breeders to modify the transition from the vegetative to the generative phase of the genotypes by changing the scale of photoperiod-sensitivity and vernalization requirements. This makes it possible to create new varieties with better adaptation for the changes of environmental conditions in the future. Based on the results, almost all of the traits examined were significantly influenced by the genotype; morphological and yield components were influenced to a greater extent by the genotype (explaining 20-58% and 50-60% of the phenotypic variance in the two trait groups). Productive tillers and grain yield were the only exemptions of this, which can be explained by the uncertainty of determinining the productive tillers in field conditions. The experiments were implemented on the same area, thus we were able to analyze in details not only the genotypic effects but also the effects of the two environmental components. Of the two environmental factors, years had generally a more significant effect on several traits compared to sowing time. Year had a significant influence on the late developmental phases (being responsible for 37-53% of the phenotypic variance), but it was an important factor in determining the plant height traits and spikelet number. Sowing time had a strong effect on the early developmental phases (explaining 50% of phenotypic variance), but in addition to this, it also influenced several yield components, such as the number of grains per spikelet, the grain number in the side tillers and the grain yield. Many authors have examined the effects of the starting date and lenght of individual phenophases on yield components in terms of photoperiod and temperature. By contrast, very little information is available on the possible correlations between environmental factors (photoperiod, temperature) and yield components in field sowing date experiments. . Based on our results, the application of different sowing dates can cause substantial differences in the growth dynamics of the wheat genotypes and in the patterns of plant developmental parameters and yield components. As the yield is fundamentally determined by the quantity of assimilates produced by the plant and their distribution among the plant organs, it is obvious that the relative lengths of the various developmental phases have a decisive influence on the yield components. This was clearly reflected by the present findings. A substantial difference existed in the length of the vegetative phase between the sowing time treatments, expressed indirectly as the time between sowing and first node appearance as characterized with both the

cumulative and the effective thermal time. The tendencies showed similarities, however in the case of effective thermal time an inverse relationship were observed between the two sowing time experiments. This finding highlights the important fact, that plants are not able to step over from the vegetative to the generative phase till the cold requirement of the genotypes is not saturated completely or the photoperiod is not inductive. Thus the lower values of effective thermal time measured in the normal autumn sowing time experiments does not mean that the genotypes had a shorter vegetative phase period. The vegetative phase was longer after normal autumn sowing which resulted in increasedtillering a larger portion of which proved to be productive. By contrast, the vegetative phase was considerably shorter in the late autumn sowing, as the plants were under the influence of a vernalizing environment shortly after sowing, resulting in the rapid initiation of stem elongation straight after the cold period. Due to the shorter vegetative phase, considerable decline could be detected in tillering and in the productive tiller number of the late-sown genotypes. The intensity of stem elongation phase had a greater effect on the grain number of the main spike (explaninig 21.6% of the phenotypic variance), while Z30 and Z31 phases had significant influences on the average thousand-kernel weight (explaining 15.4 vs. 11.8% of phenotypic variance, respectively). The length of late developmental phases showed a significant correlation with the grain number per spikelet, to a lesser extent.

3.3 Association analysis

The main developmental phases and agronomically important traits are controlled by polygenes. The regulation effects of certain genes depends on the allele type distributions in the germplasm, the mechanisms of the environmental factors and genetic × environmental (G × E) interactions. The genomic regions significantly associated with these complex traits are referred to as quantitative trait loci (QTLs).

Altogether 1374 QTL effects (marker-trait associations) were identified by GWAS, which proved to be significant at least in one environment. The QTLs of different traits showed clustering on special chromosomal regions. From these, 10 chromosome regions being significant in most of the environments were highlighted and involved in more comprehensive marker regression analysis. QTLs of Z31, Z49, last internode length and the plant height parameters were in close linkage within 84-100 cM interval of chromosome 1A. We determined close linkage between several yield component QTLs on chromosome 1B (position ~ 40-70 cM). The *PPD-B1*

gene was located to 2B between 50 and 64 cM, where we detected QTLs linked especially to developmental traits. The *PPD-D1* gene linked to 2D at the position of ~ 40 cM and QTLs of developmental traits could be mostly connected to this segment. The region between 64 and 70 cM of 4A showed close correlation mainly with QTLs of morphological traits. The segment of 4B harbouring the *RHT-B1* gene between 53 and 69 cM is linked to QTLs of morphological traits and yield components. Some QTL effects were associated with plant height on chromosome 4D around 70 cM, where the *RHT-D1* gene is located. Many authors have demonstrated the effects of *RHT-B1* (4B) and *RHT-D1* (4D) genes on plant height parameters. The *VRN-A1* gene is linked to chromosome 5A (between 40 and 60 cM). In this segment we identified QTLs of developmental, morphological traits and yield components. The region of chromosome 6A (between 50 and 56 cM) was associated with morphological trait QTLs, and the region of 6B (between 40 and 50 cM) was harboured QTLs of yield components. The identification of five known major plant developmental genes in the GWAS demonstrates the effectiveness of the method. These five genes are: *PPD-B1* (2B) and *PPD-D1* (2D) photoperiod-sensitivity genes, *RHT-B1* (4B) and *RHT-D1* (4D) dwarfing genes, as well as *VRN-A1* (5A) vernalization response gene. SNP markers, which are connected significantly to these genes have a high LOD and integrated R^2 values.

3.4 New scientific results

1. Using representative samples (683) from the winter wheat gene bank collection of MTA ATK's we composed the allele catalogue of major genes responsible for vernalization (*VRN-A1*, *VRN*-B1and *VRN-D1*) and photoperiod-sensitivity (*PPD-B1*and *PPD-D1*) in addition to determining the vernalization requirements and the photoperiod-sensitivity of the genotypes.
2. We proved, that under field conditions the alleles of *PPD-B1*, *PPD-D1* and *VRN-D1* genes significantly influence heading. As a single component, *PPD-D1* had the strongest phenotypic effect, followed by *PPD-B1* and *VRN-D1*.
3. We showed that the photoperiod-insensitive allele of *PPD-B1* gene is variable. Nine versions of the insensitive *PPD-B1a* allele were identified based on copy number, the presence of a truncated copy and the intercopy structure. Their phenotypic effects on heading were proved under field conditions. The intercopy structure type showed stronger associations with heading than the copy number.

4. We assembled the MTA-ATK winter wheat GWA panel consisting of 188 genotypes and determined its population structure and genetic diversity. Using high-density molecular genetic marker systems (KASP, DArT and 15K SNP Infinium) we collated the LD-map of 7851 markers.
5. We developed a non-destructive phenotyping method for characterizing the intensive stem elongation phase, refining the widely used Zadoks-scale phenotyping system. This method enables us to quantify not only the beginning and the end of stem elongation, but also the rate of this phase.
6. In the winter wheat GWA panel the genotypic and environmental depended correlations between the developmental patterns, morphological parameters and yielding ability were determined in a series of factorial field experiments combining 3 years and 2 sowing dates.
7. Several chromosome regions were identified by GWAS, playing a significant roles in overlapping regulation of the various developmental, morphological parameters and yield components. The most significant regions are on 1A, 1B, 2B, 2D, 4A, 4B, 4D, 5A, 6A and 6B.
8. QTL effects connected to five known plant developmental genes were also identified by GWAS demonstrating the effectiveness of the method. These five genes are *PPD-B1* (2B) and *PPD-D1* (2D) photoperiod-sensitivity genes, *RHT-B1* (4B) and *RHT-D1* (4D) dwarfing genes, and *VRN-A1* (5A) vernalization response gene. Through their complex regulation mechanisms these genes have significant influence on several morphological traits and yield components in addition to plant development.

4 CONCLUSIONS AND RECOMMENDATIONS

4.1 Genetic diversity examinations with molecular marker systems

By examining the gene bank winter wheat germplasm collection of MTA-ATK we composed a wheat gene catalogue, which contains the precise allele combinations of vernalization response (*VRN-A1*, *VRN-B1* and *VRN-D1*) and photoperiod-sensitivity (*PPD-B1* and *PPD-D1*) genes. We determined not only the allele type of these genes, but also examined how each allele affects the heading in field conditions, in Martonvásár. Significant influence of alleles of *PPD-B1*, *PPD-D1* and *VRN-D1* genes has been proven on heading, including single gene action and epistatic interaction between these genes, which together were responsible for 40% of the phenotypic variance in heading. The effects of the photoperiod-insensitive alleles of *PPD-B1* and *PPD-D1* genes wereintensified significantly, almost doubled, when the spring allele of *VRN-D1* gene was present in the genetic background. The second largest phenotypic effect was due to the allele types in the *PPD-B1* gene, which included not only the copy number but also the intercopy structure type and their interactions. In field-grown conditions the intercopy structure type had a stronger effect on heading than the copy number. With characterising newer wheat genotypes the gene catalog can be continuously upgraded. With the help of this catalogue it is possible to carry out designed crossings between genotypes with special allele combinations that could contribute to successful breeding of genotypes better able to adapt to the future changes in the environment. Our results on the phenotypic effects of *PPD-B1*, *PPD-D1* and *VRN-D1* genes can be of help to this work. The winter wheat GWA panel has a distinct population structure. The detected four sub-groups show close connection with the geographical origin. In general, one group contains American and Asian varieties, one includes Central European, one includes Hungarian and South European and one contains breeding materials from the western regions of Europe. With the knowledge of this internal structure, we are able to differentiate the true QTL effects from the false ones which could be originated from the casual coincidence of genetic components and phenotypic traits characteristic only to specific population subgroups. Based on our experiences reliability of the results can be improved using a matrix including the degrees of relationship which are calculated pairwise between the genotypes. Our LD marker map is characterized by densely and poorly covered chromosomal regions. This phenomenon can be traced back to the wheat genome characteristics and its self-pollinating nature. As the

distances of markers on the LD-map were originated from the meta-analyses of several bi-parental marker linkage maps, it can be assumed, that the majority of wheat varieties are the same in the marker poor chromosomal regions. Thus the whole genome association analyses is not restricted by this fact. The GWA panel can successfully be applied for examining the genetic components of any kind of traits. In our experiments we focused on the genetic analysis of developmental phases and some yield components, but this panel can be used for analyses of abiotic and biotic stress tolerance or even for quality parameters. A standard and high-efficiency marker system (15K SNP Infinium) was used for assembling the LD-map of the panel thus it can be further expanded involving newer varieties and genotypes. The important advantage of association analysis over conventional bi-parental mapping populations is that with it we can analyze simultaneously a much larger and more representative genetic material carrying more than two alleles at each loci. This significantly increases the probability of identifying loci which play roles in the genetic regulation of any phenotyic traits. As the allele compositions of a given variety are determined in the significant loci, further analyses can be carried out for the verification and identification of the candidate genes with the more conscious creations of special bi-parental genetic populations.

4.2 Effects of years and sowing time on plant developmental phases and yield components of the association winter wheat panel

The field experiments were carried out on the same area in every year, thus of the environmental elements we were able to analyse primarily the effects of years and sowing time in details. These two environmental factors have a different effect oneach traits groups. Seasons had generally a notable effect on morphological traits, but sowing time had a larger effect on most of the yield components. Their impacts on the developmental phases significantly changed as the plant development progressed; sowing time had a large effect on the early developmental phases, while years had a significant influence on the later developmental phases. We are planning to analyse the developmental, morphological and yield ability reactions of our winter wheat panel under a much diverse set of environments. In order to do this, in the past two years the panel has been sown not only in Martonvásár, but also along three different latitude zones of Europe, in Spain, in Germany and in England within the framework of an international cooperation. The complex analyses of data matrices collected from the four locations are in progress.

4.3 Association analysis

With GWAS analyses our aim was to examine in details the relationship between the plant development and yield components. Despite the failure to detect a direct correlation between grain yield and developmental patterns, it was proved that the given developmental parameters had a significant effect on most of the yield components. These relationships can be consistently detected. Thus the length of intensive stem elongation had a significant correlation with, the length of ear and the number of spikelets per spike as well as with the thousand kernel weight. By the contrast, the intensity of stem elongation determined mostly the grain number per spikelet. These basic trends could be measured due to the similar reactions of the majority of wheat varieties. There were however genotypes which behaved differently form the majority. As the reaction parameters of each genotype are available, varieties with correlation breaking attribution can be easily identified and included into further studies partly to elucidate the genetic regulation and partly to increase the phenotypic variance of a given trait and selecting specific genotypes.

With the GWAS analyses several chromosomal regions have been identified that play a role in often overlapping regulation of developmental, morphological parameters and yield components. It was typical, that several chromosomal regions played roles in determining more traits, which was already supported by the correlations among traits demonstrated in the phenotypic matrices. The five known plant developmental genes identified as significant factors by the analyses, demonstrate the effectiveness of the method. These five genes are *PPD-B1* (2B) and *PPD-D1* (2D) photoperiod-sensitivity genes, *RHT-B1* (4B) and *RHT-D1* (4D) dwarfing genes, as well as *VRN-A1* (5A) vernalization response gene. In addition, five other regions with complex significant QTL effects were identified on chromosomes 1A, 1B, 4A, 6A and 6B. The QTLs of 1A and 6A are correlated especially with plant height while the regions of 4A and 6B are linked to yield components. In the future our aim will be to analyse the role of these regions in details using guided crossing schemes.

MAIN PUBLICATIONS OF THE AUTHOR

Scientific publications:

Publications in international scientific journals:

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