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MAPPING OF AGRONOMIC IMPORTANT QTL IN HEXAPLOID WHEAT (*TRITICUM AESTIVUM* L.)

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Majoritatea caracterelor care prezintă interes incontestabil pentru genetica şi selecţia plantelor sunt cantitative şi, de regulă, structura alelică a câtorva locusuri sau un număr mare de gene condiţionează modul de exprimare a lor. În lucrare sunt prezentate date molecular-genetice ale analizei cantitative a locusurilor (QTL) care determină manifestarea unor caractere utile ale populaţiilor eterogene de grâu din diverse zone ecologico-geografice ale Rusiei. Pentru aceasta, în studiu au fost implicate populaţii de grâu comun de toamnă (*Triticum aestivum* L) cartate genetic. Evidenţierea mecanismelor de realizare morfobiologică a particularităţilor genetice de exprimare a caracterului moştenit şi a trăsăturilor cantitative la plantele de *T.aestivum* contribuie la evidenţierea naturii genetice a trăsăturilor cantitative şi la decodarea mecanismelor molecular-genetice de ereditate la plantele superioare care sunt influenţate de interacţiunea complexă „genotip-mediu ambiant". Cercetările ample care se vor efectua pentru identificarea şi localizarea QTL vor permite, de asemenea, să fie apreciate perspectivele de utilizare a metodologiei de cartare molecular-genetică QTL, incluzând şi aşa-numita selecţie asistată de marcheri, în diferite regiuni pedoclimaterice ale Rusiei, cu soluţionarea unor probleme de selecţie în fitotehnie şi producerea agricolă.

Genetic linkage maps offer the possibility of developi0ng genetic studies on various agronomic traits through the localization of major genes and quantitative trait loci (QTLs), as well as helping breeding programs with marker-assisted selection (MAS). In addition, positioning molecular markers on genetic map is of great help when evaluating genetic diversity of resources and establishing genetic relationships between cultivars in order to carry out the optimal breeding strategy. Homogenous distribution of markers and good genome coverage are two central desirable features of markers in this case.

Wheat (*Triticum aestivum* L.) is one of the most important food crops in the world, and understanding its genetic and genome organization using genetic maps and molecular markers is of great value for genetic and plant breeding purposes. It is an allohexaploid (2n=6x=42) with three genomes A, B, and D and has extremely large genome of 16 x 10^9 bp/1C [1] with more then 80% repetitive DNA. In the early Nineties such kind of hexaploid wheat the "International *Triticeae* Mapping Initiative" (ITMI) was used for joint mapping of RFLP [2, 3, 4, 5, 6] or SSR markers [7]. On the genetic map of ITMI wheat population up-to-now about 800 RFLP loci and 600 SSR markers have been mapped. Although molecular well-characterised recombinant inbred lines exist, only few data on using that population for trait mapping are described. For some major genes; determining red gain colour (*R1*, *R3*), red coleoptile colour (*Rc1*, *Rc3*), inhibition of epidermal waxiness ($W2^1$), kernel hardiness (*Ha*), vernalization response (*Vrn1*, *Vrn3*) or leaf rust resistance (*Lr34*), the already known map positions have been confirmed [2, 3, 4, 8, 9], whereas new QTLs were described for leaf and steam rust [2], *Pyrenophora tritici-repentis* resistance [10], Karnal bunt [11] or stripe rust [12]. Additionally a set of recombinant inbred lines of bread wheat was used for mapping of QTL associated with grain quality. It was found that loci associated with milling characteristics, rheological and mixing properties compress into three separate groups with independent genetic control [13].

In the present study a set of 110 RILs of the ITMI mapping population was evaluated for agronomical important traits under two different environments in two diverse ecological zones of Russia.

Materials and methods

The ITMI mapping population was created by crossing the spring wheat variety "Opata 85" with the synthetic hexaploid wheat "W7984", generated via a cross of the *Aegilops tauschii* Coss. accession "CIGM 86940" (2n=2x=14, DD) with the tetraploid wheat "Altar 84" (2n=4x=28, AABB). The interspecific hybridization was carried out by Dr. A. Mujeeb-Kasi (CIMMYT, Mexico). However, the results of Singh et al. (2000) [12] indicate that pedigree of the synthetic wheat may not be correct.

The ITMI mapping lines were grown in plots at two localities: Pushkin Experimental Branch of VIR (Leningrad Region) and Moscow Experimental Station (Mikhnevo, Moscow Region). In total 17 characters were evaluated in each of environments. All agronomical characters were scored according to broad unitised classifier for genera *Triticum* L. adopted at the VIR.

QTL analysis was performed with QGENE [14]. For each trait a separate QTL analysis was performed. Only loci with a LOD > 1 were taken into account.

Results and discussion

In total more that 255 records covering the 17 traits considered were analysed. Out of this 56 and 34 were found to be determined by at least one QTL having a LOD score higher than 2 and 3, respectively (Table).

The loci with a LOD score between 2 and 3 as well as between 1 and 2 will be designated as minor QTLs, the ones with LOD scores > 3 as major QTLs. For length of period from shooting to booting major as well as minor QTLs mainly contributed by Opata 85 were detected in each of environments. For length of period from shooting to flowering minor QTLs were discovered, mainly contributed by Opata 85, but major QTLs come from W7984. At the same time for length of period from shooting to heading one major QTL was inherited from W7984 (Moscow Region) and the other major QTL from Opata 85 (Leningrad Region).

Table

QTL of Different Traits Chromosome Distribution*

№	Trait	LOD (Leningrad Region)**			LOD (Moscow Region)**		
		1< ... <2	2< ... <3	3 < ...	1< ... <2	2< ... <3	3 < ...
1	Vegetative period – shooting to booting	3B, 4B, 5D	5A	5A(2)	3D, 2B(2), 5B, 5A		5A
2	Vegetative period – shooting to heading	2B, 5D	4A, 5D	5A	3A(2), 5D, 2B	7D, 5D	5D
3	Vegetative period – shooting to flowering	5B(2)	5A, 5D		2B, 1B	5D	5D(2), 7D
4	Character of flowering	3A, 1A, 5A, 2B, 3D	5D		7D, 5A, 2A(2), 3B		
5	Leaf-flag position	3D	3A(2), 1A	3A(5)	4A, 6D, 5B(2), 3D, 2B(7), 3A	3A	1B
6	Leaf-flag length	5D(2), 5A	5A(2), 2B, 1B, 5D		7D, 2D, 3A, 1B	5A	
7	Leaf-flag width	2D(2), 1D, 2B(2),	7D	5A	3D, 1A(2), 7D, 4B,		
8	Leaf waxy bloom (outer)	5B, 2A, 7B(4), 1D(2), 4D			2B, 2A	2D(3)	2D
9	Stem waxy bloom	2A(2), 2D, 6D		2D(4)	4B, 7D		6D, 2D(4)
10	Stem-length of the upper internode	7A, 2D, 6B, 2A	5A, 4B(2)	4B	4A(4), 3A(2), 5B, 5D	4A, 2B, 5B(2), 2A	
11	Stem-node size	6B, 3A, 7B	1B(2)		1D(3), 7B(2), 3D	4A, 2A	
12	Plant height	6A, 1D, 6D, 5A	4B		4D, 3A(3), 2B, 4A	3A, 2B, 4A, 2A, 1A	

13	Spike texture	6A, 7A, 5A, 6D(2), 7B, 2D, 6D			1B, 3D(3), 6A		
14	Spike waxy bloom	3A, 7B, 1D(3), 5A		2D(4)	1D, 3A, 4A, 7D	1D(2), 2B, 2D	2D(3)
15	Spike shape	3D, 2D(2), 6A, 2B			1A, 5B, 7A, 7B, 3D	1D	
16	Spike awnedness	6D, 5D, 7D, 3D(2)	1B		4A(2), 3B, 6B(2)	4A, 6B(2)	
17	Spike length	2B, 5D(2), 7B, 4A	4A(3), 5D		4A(3), 2A, 2B(2), 5A	4A(2)	4A(2)

* - In brackets the number of positions at indicated chromosome on this LOD-score occasion is given.
** - An empty cell means no data on this LOD-score occasion.

In addition to them minor QTLs were also revealed. Character of flowering is given only minor QTLs contributed mainly by Opata 85. Leaf-flag position becomes formed under the control of minor and major QTLs mainly contributed by W7984 and detected in both environments. For leaf-flag length only minor QTLs contributed also by W7984 were shown. At the same time major and minor QTLs determining leaf-flag width character were contributed by Opata 85 and shown for both localities. For stem and spike waxy bloom minor and major QTLs mainly contributed by Opata 85 were detected. As to leaf waxy bloom (outer) essentially minor QTLs were revealed. Stem-node size becomes formed by minor QTLs mainly contributed by Opata 85. The traits plant height and stem-length of the upper internode are determined by major and minor QTLs contributed by W7984 and revealed in Leningrad Region, and minor QTLs contributed by Opata 85 and detected in Moscow Region. Spike texture was formed by minor QTLs in both environments and determined QTLs mainly contributed by W7984. Practically the same we have observed for QTLs spike shape. Spike awnedness controlled only by minor QTLs contributed by Opata 85 in Moscow Region and by W7984 in Leningrad Region. Spike length becomes formed in Moscow Region by major QTLs contributed by Opata 85 and minor QTLs contributed by W7984 in Leningrad Region. Spike length become formed only by minor QTLs half of which contributed by W7984 and other half contributed by Opata 85.

The analysis of variance showed that string differences exist between genotypes for all seventeen analysed quantitative traits. The interaction with environments was much smaller. The correlation coefficients for the single traits between pairs of experiments can serve as rough estimates of heritability in these experiments. The quantitative traits formed two distinct groups. Character of flowering was highly correlated with length of vegetative period. Plant height, leaf-flag position, length, and width of leaf plate, leaf waxy bloom (outer), stem waxy bloom, stem-length of the upper internode, stem-node size, and different spike characters formed the other group of positively correlated traits. No correlation was found between these groups.

With the analysis of QTLs, critical chromosomal regions can be identified. This is the main goal of the so called AB-QTL approach [15]. With this method regions with exotic materials can be searched for, which could be of interest for transfer into breeding material. The situation in this study is quite the same. Both parents of the mapping population are exotic and not adapted to North-West and Central regions of Russia conditions. Plant breeders can use information from QTL analysis only if the results can be reproduced. It means that the experiments should be done once or twice in the same locality. However, even in this case not all QTLs could be detected in all experiments. The main reasons are the interaction between genotypes and environments, and the experimental error.

QTLs can be detected only if the parents carry different alleles. The favourable allele may be very specific for one of the parents and can not be found in other genotypes. Nevertheless, the detected QTLs indicate that an improvement is possible if chromosomal regions with positive effects are combined. As indicated above, in our experiments the quantitative traits were correlated. This resulted in QTLs for more than one trait at the same position. The data do not allow one to separate closely linked loci and pleiotropy.

In the literature a LOD of <3 is often considered as a lower value, since the QTL analysis is faced with multiple testing [16]. However, major and minor QTLs were detected at the same position in different localities for several traits. Therefore LOD values <3 should also be taken into account in our experiments.

In this study, QTLs with essential effects have been estimated. This has two reasons. First, not all traits could be evaluated in all experiments so that the results for some traits could not be compared. Second, the experiments focused on the interaction between genotypes and environments to check if results from one environment could be conclusions for other environment. The main conclusion based on the given results is that it is necessary to carry out additional experiments for establishing of QTLs role in genotype environment interaction.

References

1. Bennet M.D., Smith J.B. Nuclear DNA amounts in angiosperms // Phil. Trans. Roy. Soc. Lond. B. - 1976. - Vol.274. - P.227-274.
2. Nelson J.C., Van Deynze A.E., Autrique E., Sorrells M.E., Lu Y.H., Merlino M., Atkinson M., Leroy P. Molecular mapping of wheat. Homoeologous group 2 // Genome. - 1995a. - Vol.38. - P.516-524.
3. Nelson J.C., Van Deynzze A.E., Autrique E., Sorrells M.E., Lu Y.H., Negre S., Bernard M., Leroy P. Molecular mapping of wheat. Homoeologous group 3 // Genome. - 1995b. - Vol.38. - P.525-533.
4. Nelson J.C., Sorrells M.E., Van Deynze A.E., Lu Y.H., Atkinson M., Bernard M., Leroy P., Faris J.D., Anderson J.A. Molecular mapping of wheat: Major genes and rearrangements in homoeologous groups 4, 5, and 7 // Genetics. - 1995c. - Vol.141. - P.721-731.
5. Van Denze A.E., Dubcovsky J., Gill K.S., Nelson J.C., Sorrells M.E., Dvorak J., Gill B.S., Lagudah E.S., McCouch S.R., Appels R. Molecular-genetic maps for group 1 chromosomes of Triticeae species and their relation to chromosomes in rice and oat // Genome. - 1995. - Vol.38. - P.45-59.
6. Marino C.L., Nelson J.C., Lu Y.H., Sorrells M.E., Leroy P., Tuleen N.A., Lopes C.R., Hart G.E. Molecular genetic maps of the group 6 chromosomes of hexaploid wheat (*Triticum aestivum* L. em Thell.) // Genome. - 1996. - Vol.39. - P.359-366.
7. Röder MS., Korzun V., Wendehake K., Plashke J., Tixier M.-H., Leroy P., Ganal M.W. A microsattellite map of wheat // Genetics. - 1998. - Vol.149. - P.2007-2023.
8. Sourdille P., Perretant M.R., Chamet G., Leroy P., Gautier M.F., Joudrier P., Nelson J.C., Sorrells M.E., Bernard M. Linkage between RFLP markers and genes affecting kernel hardiness in wheat // Theor. Appl. Genet. - 1996. - Vol.93. - P.580-586.
9. Khlestkina E.K., Pestsova E.G., Röder M.S., Börner A. Molecular mapping, phenotyping expression and geographical distribution of genes determining anthocyanin pigmentation of coleoptiles in wheat (*Triticum aestivum* L.) // Theor. Appl. Genet. - 2002. - Vol.104. - P.632-637.
10. Faris J.D., Anderson J.A., Francl L.J., Jordahl J.G. RFLP mapping of tan spot resistance genes in wheat // In: McGuire P.E., Qualste C.O. (eds.). Proc. 5th, 6th Public Workshop In Triticeae Mapping Initiative. Genetic Resources Conservation Program. Division of Agriculture and Natural Resources. University of California. - 1996. - P.179.
11. Nelson J.C., Autrique J.E., Fuentes-Davila G., Sorrells M.E. Chromosomal location of genes for resistance to Karnal bunt in wheat // Crop Sci. - 1998. - Vol.38. - P.231-236.
12. Singh R.P., Nelson J.C., Sorrells M.E. Mapping Yr28 and other genes for resistance to stripe, rust in wheat // Crop Sci. - 2000. - Vol.40. - P.1148-1155.
13. Pshenichnikova T.A., Ermakova M.F., Chistyakova A.K., Shchukina L.V., Börner A., Röder M. Molecular mapping of loci associated with quality of bread wheat grain // Sel'skokhozyaistvennaya Biologia. - 2006. - Vol.5. – P.41-47.
14. Nelson J.C. QGENE: software for mapping – based genomic analysis and breeding // Mol. Breed. - 1997. - Vol.3. - P.239-245.
15. Tanksley S.D., Nelson J.C. Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines // Theor. Appl. Genet. - 1996. - Vol.92. - P.191-2003.
16. Lander E.S., Botstein D. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps // Genetics. - 1989. - Vol.129. - P.185-199.

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