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INTROGRESSIVE WHEAT LINES *TRITICUM AESTIVUM*/*AMBLYOPYRUM MUTICUM* AS TO STRUCTURE OF HOMOEOLOGOUS GROUP 5 CHROMOSOMES

Many genes for resistance to biotic and abiotic stresses have been transferred to the genomes of modern wheat cultivars from wild relatives of wheat [1]. *Amblyopyrum muticum* (Boiss) Eig (*Aegilops mutica* Boiss) is one of diploid wild relatives of wheat which is resistant to fungal diseases, and until recently was not much used for introgressive hybridization [2, 3]. Aurotica is a genome substitution amphidiploid which combines in its genome (AABBTT) tetracomponent (AABB) from common wheat winter cultivar Aurora, and T genome of *Aegilops mutica* [4]. During a 20-year period of monitoring, Aurotica demonstrated resistance to powdery mildew and rust, and additionally was characterized by higher winter hardiness. Using genome mixing method [5], wheat introgressive lines are being developed, which contain different amounts of T genome chromatin in their genomes, and are derived from the initial Aurora x Aurotica cross. Aurotica (AABBTT) is characterized by higher level of winter tolerance compared to Aurora cultivar (AABBDD), and this is supposed to be determined by genes of T genome. Freezing tolerance and winter hardiness in wheat is controlled by genes on chromosomes of homeological group 5 [6], for this reason analyzing the developed lines, first of all we tried to identify those containing introgressions in chromosomes of this group. Microsatellites (SSRs) can be used as markers for identification of alien chromatin in wheat genome. Microsatellite loci specific to chromosomes 5A, 5B, and 5D were used to identify chromatin from T genome in the genomes introgressive lines.

Materials and methods

Plant material: common wheat cultivar Aurora, genome substitution amphidiploid Aurotica, 234 42-chromosome F_5plants from crossing Aurotica and Aurora. DNA was extracted from leaves using CTAB buffer. PCR was done with primers to microsatellite loci specific to the chromosomes of homeological group 5 [7, 9, 10]. Reaction mixture contained 250 nM of each primer, 50 ng of DNA, 0,2 mM of each dNTP, 1,5 mM $MgCl_2$, 1,2 u Taq-polymerase (Fermentas). Conditions of amplification were used as recommended by primers developer. Amplification products were separated in denaturating PAAG.

Results and discussion

Hexaploids Aurora and Aurotica differ from each other by one subgenome: D genome in Aurora and T genome in Aurotica. Tetraploid component AABB in these forms must be identical. For this reason, amplification spectra for chromosomes 5A and 5B are also supposed to be identical for Aurora and Aurotica, which was true for 46 loci on chromosomes 5A and 22 loci on chromosome 5B from 62 and 29 analyzed, respectively. Primers specific to 5D chromosome must not amplify any product with DNA of Aurotica, because it does not contain subgenome D. Nevertheless, amplification product was absent only for two loci from 24 analyzed. This phenomenon could be explained by the transferability of microsatellite loci specificity among chromosomes of the same homeological group of *Triticinae*. This fact has been known from the beginning of use of microsatellite loci for mapping and comparison of *Triticinae* genomes [11]. Transferability of microsatellite loci is confirmed on our material: 8 pairs of primers from 102 analyzed were specific to two chromosomes of homeological group 5, and 3 pairs of primers were shown to be specific to all three homeological chromosomes. According to our results, transferability among D and T genomes is even higher then among other subgenomes of wheat. This could be considered as an evidence for D and T genomes homology, especially taking into account conjugation of the chromosomes from these genomes [4, 12].

Because of the transferability of the microsatellite loci among chromosomes of homeological group

5, including 5T chromosome, DNA of Aurora and Aurotica can produce identical spectra of amplification products with primers to loci specific to more than one chromosome (fig. 1).

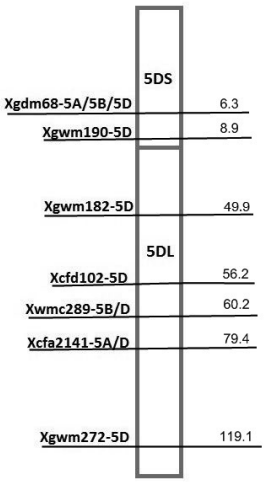

Fig. 1. Localization of analyzed microsatellite loci on 5D chromosome; distance from the distal part of the short arm of chromosome is shown in map units [7, 9, 10]

If SSR loci from T genome have alleles differing from Aurora, then specific components appear in spectra of Aurotica. It was true for microsatellite loci *Xcfa2151* (5A/5D), *Xwmc289* (B, D), *Xgdm68* (5A/5B/5D) та *Xgwm293* (A, B, D). When primers to the loci *Xwmc233, Xcfd18, Xcfd8, Xgpw5098, Xgwm292, Xcfd29, Xcfd183, Xwmc765, Xwmc443, Xgwm654* produce identical spectra with DNA of Aurora and Aurotica, this indicates that these loci are specific not only to the genome D, but also to the genome T, and alleles at these loci in Aurora and Aurotica are identical. The same explanation is true for the loci *Xcfa2104* (A, D), *Xcfa2185* (A, D), *Xbarc316* (A, D), *Xbarc232* (A, B, D).

For the identification of T genome chromosomes in the genomes of introgressive lines only 9 microsatellite loci are appropriate: *Xcfd189, Xcfd102, Xgwm190, Xgwm272, Xgwm182, Xcfa2141* (5A/5D) *Xgdm68 (A, B, D), Xgwm293 (A, B, D) Xwmc289 (B, D)*. Aurotica has specific product of amplification with the primers to these loci. The fact that four of these 9 loci produce amplification product with A and B genomes, could be theoretically ignored, because this part of the genomes of Aurora and Aurotica must be identical and differences in spectra are supposed to be caused by the presence of different alleles of these microsatellite loci in D and T genomes. Microsatellite locus *Xcfd57* does not produce amplification product with DNA of Aurotica, which could be caused by the absence (or rearrangement) of the corresponding region of 5D chromosome. This locus provides no evidence about the presence of chromosome segment from T genome.

As for 5A and 5B chromosomes, difference in spectra of amplification products between Aurora and Aurotica was observed for 12 loci on the chromosome 5A (32% from the studied loci) and for 2 loci on the chromosome 5B (14% of studied loci). Differences between Aurora and Aurotica for microsatellite loci *Xwmc752, Xwmc150, Xcfd40, Xbarc117, Xwmc805, Xbarc1, Xgwm186, Xbarc142, Xgwm179, Xcfd39, Xgwm291, Xcfd47* (chromosome 5A), and *Xgwm544, Xcfd7, Xwmc160* (chromosome 5B), can be caused by one of the three factors. First, while extracting AABB tetracomponent from the AABBDD genome of common wheat, which was done by crossing Aurora cultivar with durum wheat and six backcrosses of pentaploid hybrids with Aurora, recombination between chromosomes of A and B genomes of common and durum wheat occurred [13]. That could be the reason for partly disrupted identity between tetra-Aurora and Aurora AB genomes. Second, information about transferability of studied microsatellite loci among chromosomes of one homeological group was obtained not on Aurora, but other wheat genotypes [7, 9, 10]. It is possible, that in Aurora genome these loci are specific also to 5D chromosome, and it is also possible, that they are as well specific to chromosome 5T of Aurotica. Third, during last decade many works, including those on *Triticinae*, demonstrated that amphidiploidization process (development of Aurotica included amphidiploidization) is accompanied by numerous, and possibly directed, rearrangements in the genome, and these rearrangements include changes in microsatellite loci [14].

For screening of 234 42-chromosome F_5 plants from crossing Aurora x Aurotica 7 SSR loci with diagnostic value for identification of substitution of microsatellite locus characteristic to 5D chromosome to that specific to 5T were used (fig. 1). Aurora x Aurotica hybrid had AABBDT genome structure. Conjugation between D and T chromosomes was demonstrated before, although some univalents were also present in metaphase [4, 12]. This is confirmed by variation of chromosome numbers from 33 to 46 among F_2 offsprings of Aurora x Aurotica hybrid, which occurs because of irregular segregation of chromosomes of D and T genomes to the poles of meiocytes. Only fertile plants participated in forma-

tion of next hybrid generation, which caused narrowing of the variation of chromosome numbers, and in F_5 generation offsprings of only 15 F_2 plants were present. In generations from F_2 to F_5 plants with chromosome numbers near 42 were fertile; they were formed from gametes with almost normal chromosome constitution. For this reason it is credibly that among 15 F_2 plants whose F_5 offsprings were analyzed, ¾ of plants have at least one chromosome 5T (11 plants from 15), and ¼ have only 5D chromosomes (4 plants from 15). Comparison of amplification products spectra of F_5 plants demonstrated that 5T chromosome was present in the genomes of 13 F_2 plants, and it was absent in two plants, which corresponded to the expected chromosome numbers (according to the Fisher's exact test, P = 0,651).

If chromosomes 5D and 5T conjugate, recombination can occur between them, and among offsprings of successive generations not only plants with intact 5T chromosome, but also plants with recombinant 5D/5T chromosome may be present. According to the obtained results, it was possible to make hypothesis about chromosome constitution of plants from generations F_2–F_4 as to the presence of 5T chromosome: was it mono- or disomic substitution 5D/5T. Additionally, it was possible to identify plants with recombinant 5D/5T chromosomes. Evidence that a plant from F_2 generation had a pair of nonrecombinant chromosomes could be represented only as presence of the same (nonrecombinant) chromosome constitution in F_5 generation, and therefore in all in-between generations, which were not analyzed. Only one such plant was identified among F_2 generation. Although that plant could have another chromosome structure, it could not be demonstrated from analysis of the existent offsprings. Other 12 plants must have had two intact chromosomes, 5D or 5T, or one or both of them were recombinant and contained alleles of microsatellite loci characteristic to both chromosomes. These two cases could not be distinguished basing on our results: if F_2 plant had two intact chromosomes 5D and 5T, recombination between them could have occurred during the formation of gametes of any following generation, however only F_5 generation was analyzed. Among the plants of F_3 generation only 6 plants could possibly have nonrecombinant 5T chromosome in the genome. One of them could have disomic substitution 5D/5T, because all the analyzed F_5 offsprings of this plant were characterized by this chromosome constitution. Five other F_3 plants possibly had one intact 5T chromosome, and one chromosome with rearrangements and insignificant (three plants) or significant (one plant) inclusion of fragments from 5T chromosome. Among the offsprings of the F_3 plants, in F_5 generation 21 plants which can be considered disomic substitution lines 5D/5T were identified using microsatellite analysis. All the rest plants from F_3 and F_4 generations contained in their genomes recombinant chromosomes 5D/5T, which combined fragments of these chromosomes in different combinations.

Numerous 5D/5T recombinant chromosomes were observed among F_5 plants, and recombination events could occur in any generation beginning from F_2. This confirms hypothesis [12] about homology of D and T genomes, and indicates that T genome of *Amblyopyrum muticum* belongs to the group of *Triticinae* genomes which can conjugate with chromosomes of at least one of three subgenomes of common wheat. These genomes are very valuable for introgressive hybridization as they give the possibility to obtain introgression of small fragments of alien chromosomes into homeological chromosome of wheat through recombination process (without application of specific methods). It is important to obtain translocations from alien chromosomes, which may carry useful genes, in very small amount of alien chromatin. Identified plants with 5D/5T recombinant chromosomes are promising material for future work on introgressive hybridization and genetic analysis.

On the other hand, recombination between D and T chromosomes makes more difficult identification of alien disomic substitution lines. When D chromosomes do not conjugate with chromosomes from alien genome, it is possible to use only 1–2 biochemical markers on chromosome combined with analysis of univalent numbers in metaphase I of hybrids among recipient genotype (Aurora) and 42-chromosome introgressive line for identification of substitutions of D genome chromosomes by chromosomes of genomes S, S^{sh}, and U of three wild species: *Aegilops speltoides, Ae. sharonensis, and Ae. umbellulata*, respectively. However, when chromosomes of donor (T) and recipient (D) genomes conjugate and form recombinant chromosomes with frequency much higher that frequency of preservation of intact chromosomes in generations, identification of such chromosomes requires screening of the plant material with several marker loci per chromosome. Markers should cover both chromosome arms, and preferably on distal and proximal regions from centromere.

In electrophoretic spectra of some plants appeared such component (amplification product) that differed from both Aurora and Aurotica components, and it was designated as «new» one. Appearance of

new alleles in microsatellite loci can be explained by mutations, as their frequency is higher in genome regions with repeats (such as SSR loci). From 7 studied microsatellite loci, such «new» alleles were absent only for two loci — *Xgdm68* (A, B, D) and *Xgwm182*. New alleles appeared not only on chromosomes D and T, but also on chromosomes of A and B subgenomes.

Conclusions

Comparison of amplification products spectra produced with DNA of Aurora and Aurotica with primers to microsatellite loci specific from one to three chromosomes of homeological group 5, gave the possibility to identify loci which are diagnostically valuable for identification of plants with substitution 5D/5T and recombination 5D/5T among 42-chromosome offsprings of F_5 hybrid from Aurora x Aurotica cross. Among total 234 plants 24 plants with disomic substitution 5D/5T were identified. Numerous plants carry recombinant chromosome 5D/5T. Recombination event could have occurred in any generation beginning from F_2. Microsatellite analysis of introgressive material has some limitations because of high mutation rate in SSR loci, and potential transferability of these loci on more than one chromosome of the same homeological group. Plants with recombinant chromosomes 5D/5T are promising material for future research.

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Aims. Using SSR loci specific to homeological group 5 chromosomes, identify T genome introgressions in the genomes of F_5 wheat plants from crossing Aurora x Aurotica. ***Methods.*** DNA extraction using CTAB method, PCR with primers to SSR loci, electrophoresis in PAAG, silver staining. ***Results.*** Comparative microsatellite analysis of Aurora and Aurotica identified 9 SSR loci specific to D genome which produced differing amplicons with Aurotica DNA. These loci were used for screening (Aurora x Aurotica) F_5 progeny (234 plants); 24 plants with disomic substitutions 5D/5T were detected, and a significant number of plants containing recombinant chromosome 5D/5T. The recombination event could occur in any generation from F_2. ***Conclusions.*** Microsatellite loci could be used for identification of introgressions from *Aegilops mutica* in wheat genome (although with some limitations). Identified plants with recombinant 5D/5T chromosomes are perspective for future work.

Keywords: introgressive hybridization, *Amblyopyrum muticum*, microsatellite analysis, SSR loci, alien-substitution lines, recombinant lines.