GENETIC ANALYSIS OF ARTIFICIAL *TRITICINAE* AMPHIDIPLOID AUROTICA BASED ON THE GLAUCOUSNESS TRAIT

V.V. SHPYLCHYN, M.Z. ANTONYUK, T.K. TERNOVSKA

National University of «Kyiv-Mohyla Academy», Kyiv, Ukraine
E-mail: tern@ukma.kiev.ua

Changes in plant genomes of hybrid origin which become apparent on different levels of phenotypic manifestation of genetic and epigenetic changes are an important object of cytogenetics and molecular genetics. The changes in expression of the glaucousness trait in the artificial Triticinae amphidiploid Aurotica (AABBTT) were investigated; haplotypes of plants with the appearance of glaucous and non-glaucous genes were determined by hybridization experiments. It was supposed that the changes in expression can be explained by (epi)mutations abolishing the efficiency of dominant alleles of orthologous series of glaucous inhibitor gene Iw. Analysis of F_2 population with SSR markers specific to 2B and 2D wheat chromosomes and 2T chromosome of Aegilops mutica manifested associated inheritance of the Wms 102 and Wms 702 loci, which mapped to the short arm of chromosome 2D. The Wms702 marker was linked to the Iw2(T) gene and can now be used to detect that gene.

Keywords: *amphidiploids, genetic instability, glaucousness, microsatellite markers.*

Introduction. A waxy bloom covers the surface of all vegetative plant organs, although the expressivity of the trait can vary considerably. In particular, two gradations can be clearly distinguished in cereals: the wax cover is so heavy that the plant acquires a blue tinge and is considered to be glaucous; or the wax cover is light, the plant looks bright green, and this gradation is called 'non-glaucous'. Speaking in terms of these two gradations, their genetic control in common wheat has been established. In common wheat (*Triticum aestivum* L.), dominant alleles of the orthologous gene series, *W1* (2BS) and *W2* (2DS), are responsible for the intensive wax cover. Recessive homozygotes for *w1* and *w2* alleles are non-glaucous. In the second series of orthologous genes, *Iw1* (2BS), *Iw2* (2DS), the dominant alleles of any of the latter inhibit the effect of the dominant alleles of the first series, and no glaucousness is present. Recessive alleles *iw1, iw2* have no inhibitory effect [1−5]. Some of these genes were identified in tetraploid species of wheat with AABB

genome [6, 7]. A gene promoting the development of very heavy glaucousness was localized on chromosome 2A of *T. durum* [8]; it was also shown that chromosome 2A of Chinese Spring is also related to the development of glaucousness [9]. Another glaucousness inhibitor, *Iw3* (1BS), was discovered for *T. turgidum* [10]. The *Iw3* gene inhibits the expression of dominant promoters of glaucousness located on the group 2 chromosome in spikes and does not affect their expression in wheat leaves. According to our data, alien substitution lines where 1D chromosome in variety Aurora was substituted by a homoeolog from the S genome of *Ae. speltoides* or the S^{sh} genome of *Ae. sharonensis* were characterized by a bright green spike and glaucous leaves [11]. Thus, the spike glaucousness inhibitor is typical not only for tetraploid wheat species, but also for *Aegilops* species. The gene *Ws* was localized on chromosome 1BS of *T. dicoccoides* [12]. The authors indicated that it constitutes a spike glaucousness gene, however no other explanations were given. It is unknown whether *Iw3* and *Ws* genes are allelic, as their location on 1BS map is approximately the same.

The glaucousness/non-glaucousness trait is not selectively neutral. It was shown that wild species of *Triticinae* consist of mainly non-glaucous plants, while the cultivated cereal varieties are almost always glaucous [7]. Glaucousness is associated with an increased grain yield, especially in arid conditions [13], and increased efficiency of water use, transpiration and photosynthesis [14, 15]. However, glaucous plants are more sensitive to leaf fungal pathogens, which can be caused by their poor moistening on their surface during fungicidal treatment [16]. Moreover, non-glaucous plants demonstrate longer period of vegetative growth [7].

Variability based on the above trait was observed among artificial hexaploids of the *Triticinae* [17]. The amphidiploids Aurotica has the AABBTT genome and, in fact, is a genome-substitution amphidiploid of wheat [18], where subgenome D is

substituted by genome T of a diploid species of *Aegilops mutica* (*Amblyopyrum muticum* (Boiss.) Eig). The hexaploid was developed as an amphidiploid of the AABB tetraploid component of the Aurora variety of common winter wheat (*Triticum aestivum*, AABBDD) [19] and diploid *Aegilops mutica* [17]. Aurora variety as well as its AABB tetra-component are glaucous, while *Aegilops mutica* is non-glaucous. The newly developed amphidiploid was green, i.e., non-glaucous. Therefore, the *Aegilops* genome carries the dominant glaucousness inhibitor *Iw*. The 42-chromosome amphidiploid segregated later into three morphologically different lines: Aurotica 1, which did not differ from the initial amphidiploid, Aurotica 3 with a looser spike, and Aurotica 2 with small awn-like appendages on the spike. Aurotica 2 after several generations produced blue plants i.e., glaucous among the green ones, plants where both the spike and the leaves were glaucous. Blue plants and plants with a green spike and blue leaves (the green-blue specimen) began to appear among the green Aurotica 1 plants after eight to ten generations [17]. Thus, inexplicably, the above material demonstrated a change in the state of the glaucousness inhibitor gene from the dominant to the recessive state, with the consequent loss of its inhibitory effect.

The appearance of blue or green-blue plants among offspring of the green plants became permanent and was observed not only for Aurotica but also for other genome-substitution amphidiploids and the alien-substitution lines derived by the latter [18, 20] until the green specimens were totally lost.

Similar to common wheat, the amphidiploids Aurotica is self-pollinated. It does not form natural hybrids with common wheat during simultaneous cultivation. Artificial pollination of Aurotica with common wheat pollen produces a low percentage of F_1 hybrids, while their morphology and fertility significantly differ from those of Aurotica. That is why it is impossible to explain the appearance of recessive homozygotes by self-pollination of the possible hybrids between Aurotica and common wheat.

In recent literature, significant attention is given to the processes occurring in natural and artificial amphidiploids during formation of their genomes [21−27]. It seems that the Aurotica genome, which has an introgressive origin, possesses some process resulting in the mutation of the dominant allele into a recessive one at a rate significantly higher than the known average rate of spontaneous mutation per gene per generation.

The glaucousness/non-glaucousness trait has good expressivity, and the phenotypic assessment of plants is always unambiguous. Plant material that is an artificial amphidiploid by origin and has passed at least twenty generations is an attractive model for investigation of intra-genomic processes, which take place during stabilization of hybrid genomes, using the plant morphology trait and the microsatellite molecular genetic markers suitable for detecting chromosomal rearrangements in a genome. This manuscript presents results of a genetic analysis of various accessions of Aurotica based on the glaucousness/non-glaucousness trait and microsatellite loci localized on short arms of group 2 chromosomes of wheat homoeologs.

Materials and methods. The research uses Aurotica amphidiploids (AABBTT) contrasting with respect to the glaucousness trait: green Aurotica 1 and Aurotica 2 (non-glaucous), green-blue Aurotica 1 (non-glaucous spike, glaucous leaves), blue Aurotica 2 (glaucous). Hybrid F_1 seeds from crossing of Aurotica lines contrasted on glaucousness were obtained, cultivated and subjected to self-pollination.

Glaucousness of the crossing components and hybrids was assessed visually based on one of the three gradations: 1 − glaucous plant (blue); 2 − non-glaucous plant (green); 3 − non-glaucous spike and glaucous leaves (green-blue).

Total genomic DNA was extracted from green leaves of individual plants according to a modified method of Murray [28]. Mainly Gatersleben wheat microsatellites markers were used for the analysis. PCR procedure and fragments detection were performed as described by Röder [29]. Fragment analysis was carried out in an automated laser fluorescent sequencer (ALF-express, Amersham-Biosciences). The fragment sizes were calculated using the computer program Fragment Analyser 1.02 (Amersham Biosiences) by comparison with internal size standards.

Microsatellite analysis with primers of SSR-loci specific to 2B and 2D chromosomes of common wheat was carried out for DNA of F_3 plants derived from the combination of crosses shown in Table 1. The genotype of F_2 plants was determined based on microsatellite loci alleles for four F_3 offspring. To check correspondence between the obtained and

the expected frequencies of phenotypic classes, the χ^2 test and Fisher's exact test were used [30].

Results. The assessment of contrasting accessions and their hybrids with respect to glaucousness, the information on genetic control of the trait provided in the introduction, as well as data of amphidiploid origin allow us to suggest the following haplotypes for the lines under investigation based on genes affecting development of glaucousness (Table 1).

Genes *W1* and *iw1* should be part of Aurotica's AABB genome, while genes *W2* and *iw2* are not in this genome, since subgenome D is not present. Gene *Iw3* on chromosome 1B is represented by recessive allele *iw3*; otherwise, the Aurora variety would have had a green spike and blue leaves, which is not the case. Since the amphidiploid of Aurora's blue tetracomponent AABB and the green diploid of *Ae. mutica* is green, genome T in the chromosome of homoeologous group 2 contains the dominant gene *Iw2(T)*, a member of the homoeonymous series of common wheat orthologs. Its epistatic effect inhibits manifestation of gene *W1* located in chromosome 2B. Dominant inhibitor *Iw3*(T) is quite likely to be present in 1T chromosome, even though its manifestation is masked by a epistatic effect of gene *Iw2(T)*: hybrids F_1 derived from crossing green and green-blue plants are usually green [18]. For Aurotica, this assumption is confirmed by segregation of green-blue plants from green Aurotica 1. That is why the initial Aurotica amphidiploid should have the haplotype *iw3 W1 iw1 Iw2(T) Iw3(T)*, while the green-blue accession should have *iw3 W1 iw1 iw2(T) Iw3(T)*, differing in one gene only. The difference between green Aurotica 1 and blue Aurotica 2 is assumed to be in two genes, with the blue accession haplotype being *iw3 W1 iw1 iw2(T) iw3(T)*. The sug-

gested haplotypes were confirmed by the results of segregation analysis (Table 2). To confirm the F_2 genotypes, the assessment of the F_3 plants was performed.

The result of genetic analysis shown in Table 2 is not our first attempt to understand the genetic nature of glaucous plants appearance among the non-glaucous ones. We crossed contrasting genotypes and observed segregation in F_2 and testcrosses before. The obtained results could not be explained by either mono- or dihybrid inheritance model due to a significant increase in the «blue plants» phenotypic class as compared to the theoretically estimated numbers. Moreover, in some cases, F_1 plants derived from by crossing green (dominant trait) and blue plants, or green-blue (dominant trait) and blue plants, had a recessive phenotype. In the combinations shown in Table 1, we have proved the homozygous nature of all plants involved in production of F_1 hybrids as male or female parent lines, by observing offspring of self-pollinated plants involved in the crossing. We took the above steps because, according to our long-term observations, segregation of blue plants among offspring of the dominant phenotype was common; therefore, we assumed that the dominant plants we used as crossing components could be heterozygous and could distort the actual segregation ratio as compared to the expected one. The results of segregation in F_2 (Table 2) confirmed the assumption. When the crossing involved only homozygous plants with the dominant phenotype, the segregation ratio did not differ from the monohybrid ratio for the following pairs: green Aurotica 2 − green-blue Aurotica 2, green-blue Aurotica 2 − blue Aurotica 2, green Aurotica 1 − green-blue Aurotica 1. A segregation ratio of 12:3:1, typical for dihybrid segregation with

Table 1. **Characterization of contrast Aurotica accessions and their hybrids for the glaucousness trait**

Crossing combination	Plant phenotype*			Plant haplotype	
	♀	♂	F	♀	♂
Aurotica 1 × Aurotica 1	3	2	2	*iw3 W1 iw1 iw2(T) Iw3(T)*	*iw3 W1 iw1 Iw2(T) Iw3(T)*
Aurotica 2 × Aurotica 1	1	2	2	*iw3 W1 iw1 iw2(T) iw3(T)*	*iw3 W1 iw1 Iw2(T) Iw3(T)*
Aurotica 1 × Aurotica 2	2	1	2	*iw3 W1 iw1 Iw2(T) Iw3(T)*	*iw3 W1 iw1 iw2(T) iw3(T)*
Aurotica 2 × Aurotica 2	1	3	3	*iw3 W1 iw1 iw2(T) iw3(T)*	*iw3 W1 iw1 iw2(T) Iw3(T)*
Aurotica 2 × Aurotica 2	3	1	3	*iw3 W1 iw1 iw2(T) Iw3(T)*	*iw3 W1 iw1 iw2(T) iw3(T)*

* 1 − glaucous plant, 2 — non-glaucous plant, 3 − non-glaucous spike, glaucous leaves.
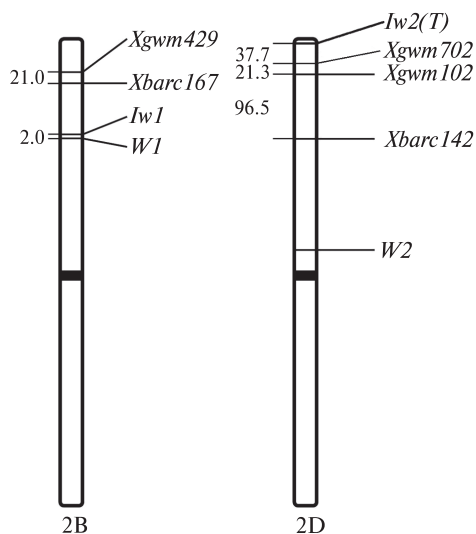
Chromosome localization of microsatellite loci under investigation and glaucousness genes. Distances are given in cM [5, 34]

dominant epistasis between genes, was observed in the crosses between green Aurotica 2 × blue Aurotica 2, and green Aurotica 1 × blue Aurotica 2.

The results of genetic analysis proved that the difference between Aurotica accessions of contrasting phenotypes is controlled by one gene for the green and the green-blue Aurotica 1 and by two genes for the green and the blue Aurotica 2. Polymorphism of contrasting plants for microsatellite loci have been studied for SSR loci mapped to the short arms of chromosomes 2B and 2D. These chromosomes were selected because glaucousness inhibitors *Iw*, epistatic both to *W1* and *W2* genes, and *Iw3(T)*, are localized there. Thus, microsatellite analysis was conducted for the population [Aurotica 1(2) × Aurotica 2(1)]F_2, which is segregated for gene *Iw2(T)*.

Microsatellite loci are considered to be one of the most efficient molecular markers due to their high level of polymorphism, which is attributed to the molecular structure of a set of repeats [31]. No SSR loci specific for the T genome have been identified to date, although, based on the haplotypes, we suggested, that the segregation is based on the genes of the T genome. We have checked 9 loci specific for the chromosome 2B. However, only one out of them (about 10 %) did not yield any

Table 2. **Frequencies of phenotypic classes in F_2 plants obtained from crossing of *Triticinae* accessions differing in glaucousness, at different developmental stages**

Crossing combinations and phenotypes (in brackets [a])	Total number of offspring	Volumes of phenotypic classes among plants which		
		assessed during heading stages	were harvested ripe	produced F_3 families and were DNA sources
Aurotica 1(3) × Aurotica 1(2)	160	70	49	27
		50(2) : 20(3)	31(2) : 18(3)	17(2) : 10(3)
χ^2 value for 3(2) : 1(1)[b] ratio	—	0.48	3.59	2.09
Aurotica 1(2) × Aurotica 2(1)	160	54	50	33
		45(2) : 5(3) : 4(1)	43(2) : 4(3) :3(1)	26(2) : 4(3) : 3(1)
χ^2 value for 12(2) : 3 (3) : 1(1)[c] ratio	—	3.21	3.89	1.26

Combinations from which DNA was not obtained

Crossing combinations and phenotypes (in brackets)	Total number of offspring	Assessed during heading stages	χ^2 and *df* value
Aurotica 1(2) × Aurotica 2(1)	120	48(2) : 5(3) : 4(1)	3.73, *df* = 2[c]
Aurotica 2(3) × Aurotica 2(1)	100	15(3) : 7(1)	0.55, *df* = 1[b]
Aurotica 2(1) × Aurotica 2(3)	200	29(3) : 13(1)	0.79, *df* = 1 [b]

[a] 1 — glaucous plant, 2 — non-glaucous plant, 3 — non-glaucous spike, glaucous leaves; [b] $\chi^2_{table\ 0.05}$ = 3,84 and $\chi^2_{table\ 0.01}$ = 6,64 for *df* = 1; [c] $\chi^2_{table\ 0.05}$ = 5,99 and $\chi^2_{table\ 0.01}$ = 9,21 for *df* = 2.

product, and only two — *Wms 429* and *Barc 167* — were polymorphic for the contrasting parent lines. No products were obtained with 7 pairs of primers out of the 18 loci specific to the 2D chromosome (about 40 %). At the same time 3 loci — *Barc 142, Wms 102* and *Wms 702* proved to be polymorphic. The latter result is consistent with the absence of the D genome in plants and confirms homoeology between the T and D genomes, which is also proved by the regular production of bivalents between the chromosomes of the two genomes [32, 33].

The viability of F_2 offspring derived from crosses of different Aurotica accessions is significantly lower than that of common wheat. From among the F_2 offspring from crosses of different Aurotica accessions only 21—48 % reached heading stage and only 68—93 % of them were harvested (Table 2). Especially low viability was demonstrated by hybrids obtained by crosses of Aurotica 2 plants with contrast phenotype.

A check of each of the five polymorphic loci for the correspondence to monogenic segregation among F_2 plants of the hybrid green Aurotica 1 × × blue Aurotica 2 shows that all five out of the SSR loci are selectively neutral and can be used to check their relation to the trait of interest (Table 3).

The distribution of F_2 offspring based on the allelic combinations of the two microsatellite loci specific for chromosome 2B proves that parental allele combinations prevail over the recombinant ones: 5 plants with 208—254 haplotype and 8 plants with 206—260 haplotype vs. two plants estimated theoretically. The above estimation corresponds to the factual localization of the above loci on the chromosome 2B (Figure).

Two genes participating in the glaucousness control, *W1* and *Iw1*, are located within a small distance from the *Wms 429* and *Barc 167* loci. However, the parental accession haplotypes suggested by us do not provide for the segregation based on the said genes, as they are the same for the above. Haplotype 206—260 for the loci of chromosome 2 is characteristic of the glaucous Aurotica 2. If the segregation based on the glaucousness/non-glaucousness trait is really unrelated to the *Wms 429* and *Barc 167* loci, the theoretic estimate for F_2 plants of the said haplotype is 6 green and 2 blue plants, while the actual outcome was 5 green and 3 blue plants, which does not differ from the estimates (according to exact Fisher test, $P_{init} = 0,4$,

P = 1,0). Therefore, no segregation based on the *W1* and *Iw1* genes occurs, which confirms the parent plant haplotypes suggested by us.

Amplification of Aurotica DNA with AABBTT genome (and without the D genome) occurs with primers that are specific for the chromosome 2D. Considering the well-documented transferability phenomenon for various *Triticinae* genomes [35—38], it is quite reasonable to assume that *Barc 142, Wms 102* and *Wms 702* loci are specific not only for the 2D chromosome, but for the 2T as well. However, possible amplification of the 2B chromosome DNA with the primers of the loci specific for the 2D chromosome should be taken into consideration. In this case, considering the chromosome localization of five microsatellite loci under study (Figure), one should expect the associated inheritance of alleles of the microsatellite loci specific for the 2B and 2D chromosomes. The study of F_2 plants in terms of co-segregation by the alleles of the microsatellite loci specific for the 2B and 2D chromosomes shows that they are inherited independently from each other. This directly proves that the amplification with the primers to the 2D chromosome loci occurs with the DNA of the 2T, and not the 2B, chromosome.

A check for independent inheritance of three microsatellite loci located on the 2T chromosome revealed no association between the *Barc 142* and *Wms 102*, or *Barc 142* and *Wms 702* loci, while the allele inheritance of *Wms 102* and *Wms 702* loci in parental combinations prevails over their independent inheritance. There were five F_2 plants with 203/147 typical for the green Aurotica 1, and 4 plants with 195/144 haplotype typical for the blue Aurotica 2, instead of the 4 plants according to the

Table 3. **Segregation by microsatellite loci alleles in the population (green Aurotica 1 × blue Aurotica 2) F_2**

Locus and its alleles (bp)	Number of plants with the genotype			χ^2 for 1:2:1 ratio
	aa	*ab*	*Bb*	
Wms 429 208(a), 206(b)	7	15	11	1.24 *
Barc 167 254(a), 260(b)	10	15	8	0.52 *
Barc 142 272(a), 268(b)	9	14	10	0.82 *
Wms 102 203(a), 195(b)	6	19	8	1.00 *
Wms 702 147(a), 144(b)	5	18	10	1.79 *

* P > 0.05.

theoretical estimates for both cases. The above loci are located next to each other on the 2D chromosome map, and this location is maintained on the 2T chromosome.

Out of the two genes − *Iw2(T)* and *Iw3(T)* − on which the segregation is based, according to the suggested parent accession haplotypes, we can test for association with microsatellite alleles only the *Iw2(T)* gene, as the *Iw3(T)* gene has to be located on the 1T chromosome which we did not study using microsatellites. Based on *Wms 102* locus, allele 195 is typical for the blue Aurotica 2. Out of the 8 F_2 plants homozygous for this allele, there were 6 green and 2 blue and green-blue plants formed by the *iw2(T) iw2(T)* genotype, as it should be the case during independent inheritance of the *Wms 102* and *Iw2(T)* loci (according to Fisher's exact test, $P_{init} = 0,4$, $P = 1,0$). Out of the 10 F_2 plants homozygous for allele 268 of locus *Barc 142* typical for the same line, there were 6 green and 4 blue and green-blue plants, which did not differ from the theoretical estimates of 8 and 2, respectively (according to Fisher's exact test, $P_{init} = 0,2$, $P = 0,628$). Out of the 10 F_2 plants homozygous for allele 144 of locus *Wms 702* typical for the blue Aurotica 2, there were 3 green and 7 blue and green-blue plants versus 8 and 2, respectively, based on the theoretical estimate (according to Fisher's exact test, $P_{init} = 0,032$, $P = 0,047$, the difference being statistically significant). Therefore, gene *Iw2(T)* and locus *Wms 702* are not characterized by independent inheritance and can be linked on chromosome 2T.

Discussion. According to results of genetic analysis, the green-blue phenotype hypostatic to the effect of *Iw2(T)* gene has to be produced from the self-pollination of *W1W1 Iw2(T)iw2(T) Iw3(T) Iw3(T)* monoheterozygotes or *W1W1 Iw2(T) iw2(T) Iw3(T)iw3(T)* diheterozygotes, and is characterized by *W1W1 iw2(T)iw2(T) Iw3(T)* genotype. Thus, a single mutation, *Iw2(T)→iw2(T)*, with a subsequent self-pollination, is sufficient for the green-blue plants to appear, while the *Iw3(T)* gene can remain intact. The blue Aurotica 2 plants appear in the green Aurotica 2 population due to self-pollination of diheterozygous green Aurotica 2 *W1W1 Iw2(T)iw2(T) Iw3(T)iw3(T)*. Blue plants appear among the offspring of green Aurotica 2 regularly, although this requires simultaneous mutation in two genes. The blue plants can also appear among the offspring of heterozygous green-blue plant *W1W1 iw2(*T*)iw Iw3(T)iw3(T)*. The rate of blue Aurotica 2 occurrence among the offspring of the green Aurotica 2 demonstrates a large number of diheterozygous plants among the latter. The simultaneous mutation in two different genes cannot be explained from the position of random mutagenesis. Over the past 10 to 15 years, a number of literature sources have proven that plant genomes whose origin is associated with allopolyploidization or wide hybridization are prone to some processes that could be interpreted as destabilization of such genomes [24, 25, 27]. On the phenotypic level, this means the emergence of new traits which were not present in the parent lines [39], new gene alleles of storage proteins [40, 41], and material changes in the gene expression [42, 43], right up to gene silencing [44]. The genome of Aurotica joints subgenome A and B of the common wheat genome and the T genome of diploid *Aegilops mutica*, and can also be characterized by certain molecular events causing its hybrid origin.

The results of genetic analysis provide a statistical proof that there were no differences between the empirical ratios of phenotypic class sizes and the theoretical ratios estimated based on the suggested genotypes. However, at all stages of assessment, the number of blue plants was always larger than the expected value, and the number of plants with dominant phenotype was always smaller in all of the crossing combinations (Table 4).

A similar excess of blue plants among F_2 progeny from crosses between the green and the blue wheat accessions of wheat was observed earlier during the genetic analysis of the so-called genome-addition hexaploid lines of wheat with genomes of hybrid origin. On the one hand, there were the usual amphidiploids of F_1 hybrids derived from crossing of *T. durum* Desf. and diploid species of *Ae. tauschii*, with the resulting AABBDD genome (MIT-lines). These amphidiploids were non-glaucous, i.e., green. On the other hand, there were the plants that combined the same genome of durum wheat and the D subgenome of one of the common wheat varieties. Their origin was complicated and included several hybridization events. First, a 35-chromosome hybrid between the common wheat and the durum wheat with AABBD genome was obtained. It was then crossed with durum wheat. The number of chromosomes in the offspring varied from 28 (AABB) to 35 (AABBD). Cytological control was

used to select 35-chromosome plants, which were then back-crossed with durum wheat five times. As a result, the allopolyploids were obtained where the AABB subgenome of common wheat was substituted by the AABB genome of durum wheat, while DD genome remained unchanged [45]. All of the above plants were glaucous. The green and blue genome-addition lines were subjected to genetic analysis and monogenic inheritance of the trait was ascertained. However, we revealed an excess of blue plants in F_2 and test crosses for all of the crossing combinations, which did not distort statistically the segregation ratios typical for the monogenic difference between contrasting genotypes, but attracted our attention nevertheless [46, 47]. Moreover, several generations after the green amphidiploids were formed; their blue analogues began to appear among the above. Amphidiploids were self-pollinated and their spike morphology significantly differed from the common wheat varieties as well as from the blue genome-addition lines with the D subgenome of common wheat. The study of gliadin spectra of the green accessions and their blue analogues allowed us to exclude possible uncontrolled cross-pollination of green plants. A total of seven amphidiploids with various accessions of *Aegilops tauschii* were obtained [17]. Only one out of them, MIT[346], failed to produce any blue lines so far, while blue accessions among the offspring of MIT[28] first appeared only three years ago. This phenomenon was first observed in the early 90s, several generations after the amphidiploids were formed. Thus, for amphidiploid MIT[334] the green analogue has been lost, and only blue plants are present. According to the results of hybridological analysis, the green coloration of MIT plants is associated with dominant allele *Iw2* located on chromosome 2D of *Aegilops tauschii* [46]. Similar to the case of genome-substitution Aurotica amphidiploid, the appearance of blue analogues among the artificially produced green wheat lines can be explained only by the mutation of *Iw2* allele into *iw2* allele. It is still unclear why the mutation rate is so high or why the mutation is typical for the genomes of hybrid origin.

For the crossing combinations provided in Table 1, the homozygosity of plants with a dominant genotype that participated in the production of F_1 was controlled based on the offspring derived from self-pollination of individual plants. Only the F_1 plants from homozygotes were used to produce F_2 plants. Among the F_1 plants, no segregation of the recessive phenotype was observed, although the overall number of F_1 hybrids derived from four in the green-blue Aurotica 2 × blue Aurotica 2 combination to 15 in the green Aurotica 1 × blue Aurotica 2 combination was small. Thus, no F_1 plants were home to the mutation from the dominant allele to the recessive one. The number of F_2 plants was much higher (Table 2). We have the data on estimation of two crossing combinations as for the trait of interest at

Table 4. **Empirical and theoretical numbers of phenotypic classes in F_2 plants obtained from the crossing of Aurotica accessions contrasted by glaucousness**

Crossing combinations and phenotypes (in brackets[a])	Ration between phenotypic classes among the plants which		
	assessed during heading stages	were harvested ripe	produced F_3 families and were DNA sources
Aurotica 1(3) × Aurotica 1(2)	E[b]: 53(2) + 17(3) O: 50(2) + 20(3)	E: 37(2) + 12(3) O: 31(2) + 18(3)	E: 20(2) + 7(2) O: 17(2) + 10(3)
Aurotica 1(2) × Aurotica 2(1)	E: 48(2) + 12(3) + 4(1) O: 53(2) + 6(3) + 5(1)	E: 46(2) + 11(3) + 4(1) O: 50(2) + 6(3) + 5(1)	E: 33(2) + 8(3) + 3(1) O: 35(2) + 5(3) + 4(1)
Aurotica 1(2) × Aurotica 2(1)	E: 50(2) + 13(3) + 4(1) O: 56(2) + 6(3) + 5(1)		
Aurotica 2(3) × Aurotica 2(1)	E: 17(3) + 5(1) O: 15(3) + 7(1)		
Aurotica 2(1) × Aurotica 2(3)	E: 32(3) + 10(1) O: 29(3) + 13(1)		

[a] 1 − glaucous plant, 2 — non-glaucous plant, 3 − non-glaucous spike, glaucous leaves; [b] E − expected class size, O − observed class size.

various stages of their ontogenesis: during heading, for ripe plants, and for plants which proved to be fertile enough to produce F_3 families. The crossing combination green Aurotica 1 × blue Aurotica 2 demonstrates that a decrease in the number of the assessed plants from the first to the third assessment is almost exclusively on account of green plants (Table 4). It could have been assumed that the excess of blue plants could be explained by their higher viability as compared to the green plants. In this case, the excess of blue plants in F_2 and BC_a generations could easily be attributed to the result of negative selection against the less fit green plants, which occurs during ontogenesis from germination to a ripe stage. In all of the previous studies [46, 47], the trait was assessed only during the ripening stage, the moment of maximum realization of the blue plants' adaptability, if any. Literature on the subject expresses the commonly held view on the glaucousness trait in terms of its adaptive significance. It is believed that glaucousness protects the plant from radiation, reduces the leaf temperature, increases the water use efficiency and, under certain conditions, increases the yield of barley and wheat [15, 48, 49], although, under different conditions, glaucousness demonstrated a negative effect on the productivity parameters [7, 50] and resistance to powdery mildew [51]. According to our data, in the second combination, green-blue Aurotica 1 × green Aurotica 1, a reduction in the number of green and blue plants was in proportion to their output quantities, which does not support the assumption about the higher adaptive value of blue plants. The occurrence of blue plants which we discovered among the F_1 hybrids derived from crosses between the green and the blue introgression lines of common wheat, despite the fact that such plants should not have appeared, is a serious argument against the assumption that the increase in the size of the blue plant class in segregating populations is due to their higher adaptability as compared to the green plants [20]. Therefore, the increase in the number of blue plants against the theoretically expected number should be attributed primarily to some molecular event that suppresses efficiency of the dominant alleles in the *Iw* orthologous series.

The common wheat genome contains two identified genes inhibiting the development of glaucousness in the whole plant with qualitative expression: *Iw1* on chromosome 2B and *Iw2* on chromosome 2D [5]. The genes, which ensure quantitative impact on the trait, have been localized on other common wheat chromosomes as well: 3A [52], 5A [53], 1A, 1D, 2DL, 4A, 5B, 6A, 7A, 7D [54, 55]. The plant material used for the research was unique because the crossing involved genotypes differing only by the alleles of the gene of interest due to the new mutation of the said gene, with the phenotype change being of qualitative, alternative nature. Among the two genes of the quality trait expression, *Iw1* gene is present in Aurotica genome in the recessive state, since tetra-Aurora (AABB) is glaucous. Gene *Iw2* is not present in the Aurotica genome because it does not have the D genome. Considering the genome origin and structure in various Aurotica lines, as well as their haplotypes for genes of *W* and *Iw* orthologous series, we believe that segregation is based on the gene located in the T genome, which we have labeled as *Iw2(T)*, since T genome has replaced the D genome of common wheat. The presence of members of the *Iw* orthologous series is typical for other *Triticinae* representatives as well. The location of *Iw2* gene on the distal part of the short arm of chromosome 2D was demonstrated for *Ae. tauschii* [16]. Its ortholog has been localized on chromosome 2 of *Leymus* allotetraploid species (*Leymus* wildryes) [56]. The introgression of the gene which is homologous to gene *Iw1* and, possibly, gene *Iw3* from *Ae. speltoides* to the common wheat genome has been proven [57]. Glaucousness gene *wa1* has been localized on rye chromosome 7RL. The presence of orthologous genes *gs1, gs6, gs8* was demonstrated on 2HS chromosome for barley, while gene *gl2* was demonstrated for maize [58].

Therefore, the results of the research show that mutation of the dominant gene in *Iw* orthologous series to the recessive allele causes a permanent change in the genotypic manifestation of glaucousness/non-glaucousness trait for plants of Aurotica genome-substitution amphidiploids. The mutation rate is significantly higher than the general estimate for the spontaneous mutagenesis rate. The mechanism causing the increase in the mutation rate has yet to be explained. In the light of contemporary research on ontogenetic instability of genomes, further research of the above mechanism could focus on the effect of retrotransposons [22, 59], intrachromosomal rearrangement due to illegitimate or unequal crossing-over [41, 60], as well as changes in the gene expression, including epigenetic changes [44, 61].

ГЕНЕТИЧЕСКИЙ АНАЛИЗ ИСКУССТВЕННОГО АМФИДИПЛОИДА АВРОТИКА (*TRITICINAE*) ПО ПРИЗНАКУ ВОСКОВОЙ НАЛЕТ

В.В. Шпыльчин, М.З. Антонюк, Т.К. Терновская

Изменения в геноме растений гибридного происхождения, которые проявляются на разных фенотипических уровнях реализации генетических и эпигенетических изменений, интенсивно исследуются цитогенетикой и молекулярной генетикой. Изучали изменения экспрессии признака наличие/отсутствие воскового налета у искусственного амфидиплоида Авротика (*Triticinae*, AABBTT). Гибридологическим анализом установлены гаплотипы растений с контрастными фенотипами и показано, что изменение экспрессии признака связано с (эпи)мутацией, которая приводит к утрате активности доминантного аллеля ортологической серии генов *Iw* — ингибиторов воскового налета у пшеницевых. Микросателлитный анализ расщепляющихся популяций по SSR-локусам, специфичным для 2B и 2D хромосом пшеницы и 2T хромосомы эгилопса, показал сцепленное наследование локусов *Wms 102* и *Wms 702*, локализованных на плече 2DS, а локус *Wms 702* сцеплен с геном *Iw2(T)* и может быть использован для определения этого гена.

ГЕНЕТИЧНИЙ АНАЛІЗ ШТУЧНОГО АМФІДИПЛОЇДА АВРОТИКА (*TRITICINAE*) ЗА ОЗНАКОЮ ВОСКОВА ОСУГА

В.В. Шпильчин, М.З. Антонюк, Т.К. Терновська

Зміни у геномі рослин гібридного походження, які виявляються на різних фенотипних рівнях реалізації генетичних та епігенетичних змін, інтенсивно досліджуються цитогенетикою та молекулярною генетикою. Вивчали зміни експресії ознаки наявність/відсутність воскової осуги у штучного амфідиплоїда Авротика (*Triticinae*, AABBTT). Гібридологічним аналізом встановлено гаплотипи рослин з контрастними фенотипами та показано, що зміна експресії ознаки пов'язана з (епі)мутацією, яка призводить до втрати активності домінантного алеля ортологічної серії генів *Iw* — інгібіторів воскової осуги у пшеницевих. Мікросателітний аналіз популяцій, що розщеплюються, за SSR-локусами, специфічними для 2B та 2D хромосом пшениці та 2T хромосоми егілопсу, показав зчеплене успадкування локусів *Wms 102* та *Wms 702*, локалізованих на плечі 2DS, а локус *Wms 702* зчеплений з геном *Iw2(T)* та може бути використаний для визначення цього гена.

REFERENCES

1. *Tsunewaki K.* Monosomic analysis of synthesized hexaploid wheat // Jap. J. Genet. − 1962. − **37**. − P. 155−168.
2. *Tsunewaki K.* Comparative gene analysis of common wheat and its ancestral species. 2. Waxiness, growth habit and awnedness // Jap. J. Bot. − 1966. − **19**. − P. 175−254.
3. *Rowland G.G., Kerber E.R.* Telocentric mapping in hexaploid wheat of genes for leaf rust resistance and other characters derived from *Aegilops squarrosa* // Can. J. Genet. Cytol. − 1974. − **16**. − P. 137−144.
4. *Nelson J.C., van Deynze A.E., Autrique E. et al.* Molecular mapping of wheat: homoeologous group 2 // Genome. − 1995. − **38**. − P. 516−524.
5. *Tsunewaki K., Ebana K.* Production of near-isogenic lines of common wheat for glaucousness and genetics basis of the trait clarified by their use // Genes. Genet. Syst. − 1999. − **74**. − P. 33−41.
6. *Goncharov N.P.* Location of the gene controlling the "non-glaucousness" of vegetative organs in tetraploid wheat // Russian J. Genet. − 1994. − **30**. − P. 1282−1283.
7. *Simmonds J.R., Fish L.J., Leverington-Waite M.A. et al.* Mapping of a gene (*Vir*) for a non-glaucous, viridescent phenotype in bread wheat derived from *Triticum dicoccoides*, and its association with yield variation // Euphytica. − 2008. − **159**. − P. 333−341.
8. *Allan R.E., Vogel O.A.* F_1 monosomic analyses involving a smooth-awn durum wheat // Wheat Inf. Serv. − 1960. — **11**. − P. 3−4.
9. *Tsunewaki K.* Genetic studies of a 6*x*-derivative from an 8*x* triticale // Can. J. Genet. Cytol. − 1964. − **6**. − P. 1−11.
10. *Dubcovsky J., Echaide M., Giancola S. et al.* Seed-storage-protein loci in RFLP maps of diploid, tetraploid, and hexaploid wheat // Theor. Appl. Genet. − 1997. − **95**. − P. 1169−1180.
11. *Antonyuk M.Z., Ternovskaya T.K.* Plant morphology characters as markers of the chromosome homoeologous groups of *Triticeae* // Cytology and Genetics. − 1997. − **31**, № 4. − P. 105−112.
12. *Peng J., Korol A.B., Fahima T. et al.* Molecular genetic maps in wild emmer wheat, *Triticum dicoccoides*: genome-wide coverage, massive negative interference, and putative quasilinkage // Genome Res. − 2000. − **10**. − P. 1509−1531.

13. *Monneveux P., Reynolds M.P., Gonzalez-Santoyo H. et al.* Relationships between grain yield, flag leaf morphology, carbon isotope discrimination and ash content in irrigated wheat // J. Agron. Crop Sci. − 2004. − **190**. − P. 395−401.

14. *Johnson D.A., Richards R.A., Turner N.C.* Yield, water relations, gas exchange, and surface reflectance of near-isogenic wheat lines differing in glaucousness // Crop Sci. − 1983. − **23**. − P. 318−328.

15. *Richards R.A., Rawson H.M., Johnson D.A.* Glaucousness in wheat: its development and effect on water-use efficiency gas exchange and photosynthetic tissue temperature // Aust. J. Plant Physiol. − 1986. − **13**. − P. 465−473.

16. *Watanabe N., Takesada N., Shibata Y., Bam T.* Genetic mapping of the genes for glaucous leaf and tough rachis in *Aegilops tauschii*, the D-genome progenitor of wheat // Euphytica. − 2005. − **144**. − P. 119−123.

17. *Ternovskaya T.K.* Reconstruction of common wheat (*Triticum aestivum* L.) genome for wheat genetical analysis and genes introgression : Dis. Doctor of Biol. Sci. − Kyiv, 1999.

18. *Shpylchyn V.V., Ternovska T.K.* Variation in glaucousness appearance in generations of amphidiploids of the *Triticinae* subtribe // Naukovi zapysky NaUKMA, Biology and Ecology. − 2011. − **119**. − P. 3−7.

19. *Ternovskaya T.K., Zhirov E.G.* Separation of the AABB tetraploid component out of common wheat variety Saratovskaya 29 // Dokl. VASCHNIL. − 1979. − **3**. − P. 8−10.

20. *Shpylchyn V.V., Antonyuk M.Z., Ternovska T.K.* Phenotypic polymorphism for glaucousness in accessions of the *Triticinae* subtribe // Naukovi zapysky NaUKMA, Biology and Ecology. − 2010. − **106**. − P. 3−8.

21. *Liu B., Xu Ch., Zhao N. et al.* Rapid genomic changes in polyploid wheat and related species: implications for genome evolution and genetic improvement // J. Genet. Genom. − 2009. − **36**. − P. 519−528.

22. *Bento M., Gustafson P., Viegas W., Silva M.* Genome merger: from sequence rearrangements in triticale to their elimination in wheat-rye addition lines // Theor. Appl. Genet. − 2010. − **121**. − P. 489−497.

23. *Tiwari V.K., Rawat N., Neelam K. et al.* Random chromosome elimination in synthetic *Triticum−Aegilops* amphiploids leads to development of a stable partial amphiploid with high grain micro- and macronutrient content and powdery mildew resistance // Genome. − 2010. − **53**, № 12. − P. 1053−1065.

24. *Zhao N., Xu L., Zhu B. et al.* Chromosomal and genome-wide molecular changes associated with initial stages of allohexaploidization in wheat can be transit and incidental // Genome. − 2011. − **54**, № 8. − P. 692−699.

25. *Madlung A., Wendel J.F.* Genetic and epigenetic aspects of polyploid evolution in plants // Cytogenet. Genome. Res. − 2013. − **140**. − P. 270−285.

26. *Bartoš J., Vlček C., Choulet F. et al.* Intraspecific sequence comparisons reveal similar rates of non-collinear gene insertion in the B and D genomes of bread wheat // BMC Plant Biol. − 2012. − **12**. − P. 155.

27. *Sarilar V., Palacios P.M., Rousselet A. et al.* Allopolyploidy has a moderate impact on restructuring at three contrasting transposable element insertion sites in resynthesized *Brassica napus* allotetraploids // New Phytol. − 2013. − **198**, № 2. − P. 593−604.

28. *Murray H.G., Thompson W.F.* Rapid isolation of high molecular weight DNA // Nucl. Acids Res. − 1980. − **8**. − P. 4321−4325.

29. *Röder M.S., Korzun V., Wendehake K. et al.* A microsatellite map of wheat // Genetics. − 1998. − **149**. − P. 2007−2023.

30. *Glanz S.A.* Primer of Biostatistics. − McGraw-Hill publ., 2002. − 496 p.

31. *Li Y.-C., Korol A.B., Fahima T., Nevo E.* Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review // Mol. Ecol. − 2002. − **11**. − P. 2453−2465.

32. *Jones J.K., Majisu B.N.* The homoeology of *Aegilops mutica* chromosomes // Can. J. Genet. Cytol. − 1968. − **10**, № 3. − P. 620−626.

33. *Ohta S.* Phylogenetic relationship of *Aegilops mutica* Boiss. with the diploid species of concerning *Aegilops − Triticum* complex, based on the new method of genome analysis using its B-chromosomes // Memoirs of the College of Agriculture, Kyoto Univ., Japan. − 1991. − **137**. − P. 116−120.

34. *Ganal M.W., Röder M.S.* Microsatellite and SNP markers in wheat breeding // Genomics Assisted Crop Improvement. Vol. 2. Genomic Applications in Crops / Eds R.K. Varshney, R. Tuberosa. − Springer, 2007. − P. 1−24.

35. *Sourdille P., Tavaud M., Charmet G., Bernard M.* Transferability of wheat microsatellites to diploid *Triticeae* species carrying the A, B and D genomes // Theor. Appl. Genet. − 2001. − **103**. − P. 346−352.

36. *Adonina I.G., Salina E.A., Pestsova E.G., Röder M.S.* Transferability of wheat microsatellites to diploid *Aegilops* species and determination of chromosomal localizations of microsatellites in the S genome // Genome. − 2005. − **48**. − P. 959−970.

37. *Leonova I.N., Röder M.S., Nasyrova F.* The application of wheat microsatellite markers for the detection of interspecific variation in tetraploid *Aegilops* species with C and U genomes // Cereal Res. Communs. − 2009. − **37**, № 3. − P. 335−343.

38. *Castillo A., Budak H., Martín A.C. et al.* Interspecies and intergenus transferability of barley and wheat D-

genome microsatellite markers // Ann. Appl. Biol. − 2010. − **156**. − P. 347−356.

39. *Gaeta R.T., Pires J.Ch., Iniguez-Luy F. et al.* Genomic changes in resynthesized *Brassica napus* and their effect on gene expression and phenotype // Plant Cell. − 2007. − **19.** − P. 3403−3417.

40. *Liu Sh., Zhao Sh., Chen F., Xia G.* Generation of novel high quality HMW-GS genes in two introgression lines of *Triticum aestivum/Agropyron elongatum* // BMC Evol. Biol. − 2007. − **7**. − P. 76−85.

41. *Yuan Zh., Liu D., Zhang L. et al.* Mitotic illegitimate recombination is a mechanism for novel changes in high-molecular-weight glutenin subunits in wheat-rye hybrids // PLoS One. − 2011. − **6**, № 8. − e23511.

42. *Osborn T.C., Pires J.Ch., Birchler J.A. et al.* Understanding mechanisms of novel gene expression in polyploids // Trends Genet. − 2003. −**19**, № 3. − P. 141−147.

43. *Schranz M.E., Osborn T.C.* De novo variation in life-history traits and responses to growth conditions of resynthesized polyploid *Brassica napus* (*Brassicaceae*) // Amer. J. Bot. − 2004. − **91**. − P. 174−183.

44. *Adams K.L., Percifield R., Wendel J.F.* Organ-specific silencing of duplicated genes in a newly synthesized cotton allotetraploid // Genetics. − 2004. −**168**. − P. 2217−2226.

45. *Zhirov E.G., Ternovskaya T.K.* Transfer of D genome from common wheat to durum wheat // Wheat Inf. Serv. − 1988. − **65**. − P. 4−6.

46. *Ternovskaya T.K., Zhirov E.G.* Common wheat genome D. Genetic control of glaucousness, glume hairiness, and ripe ear colour // Cytology and Genetics. − 1993. − **27**. − P. 15−20.

47. *Ternovskaya T.K.* Common wheat genome D. Inheritance of some spike morphology characters // Cytology and Genetics. − 1997. − **31**, № 4. − P. 11−18.

48. *King R.W., von Wettstein-Knowles P.* Epicuticular waxes and regulation of ear wetting and pre-harvest sprouting in barley and wheat // Euphytica. − 2000. − **112**. − P. 157−166.

49. *Gonzalez A., Ayerbe L.* Effect of terminal water stress on leaf epicuticular wax load, residual transpiration and grain yield in barley // Euphytica. − 2010. − **172**. − P. 341−349.

50. *Merah O., Deleens E., Souyris I., Monneveux P.* Effect of glaucousness on carbon isotope discrimination and grain yield in durum wheat // J. Agron. Crop Sci. − 2000. − **185**. − P. 259−265.

51. *Shpylchyn V., Martynenko V., Ternovska T.* Genetic instability of amphidiploid Miosa (*Triticum durum* ×

× *Aegilops comosa*, AABBM^tM^t) resistant to powdery mildew // Disease Risk and Food Security : Proc. 13th Int. cereal rust and powdery mildews conf. − Beijing, 2012. − P. 164−165.

52. *Bennett D., Izanloo A., Edwards J. et al.* Identification of novel quantitative trait loci for days to ear emergence and flag leaf glaucousness in a bread wheat (*Triticum aestivum* L.) population adapted to southern Australian conditions // Theor. Appl. Genet. − 2012. − **124**. − P. 697−711.

53. *Mason R.E., Mondal S., Beecher F.W. et al.* QTL associated with heat susceptibility index in wheat (*Triticum aestivum* L.) under short-term reproductive stage heat stress // Euphytica. − 2010. − **174**. − P. 423−436.

54. *Börner A., Schumann E., Furste A. et al.* Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.) // Theor. Appl. Genet. − 2002. − **105**. − P. 921−936.

55. *Kulwal P.L., Roy J.K., Balyan H.S., Gupta P.K.* QTL mapping for growth and leaf characters in bread wheat // Plant Sci. − 2003. − **164**. − P. 267−277.

56. *Larson S.R., Kishii M., Tsujimoto H. et al.* EST linkage maps identify 4NsL−5NsL reciprocal translocation, wheat-*Leymus* chromosome introgressions, and functionally important gene loci // Theor. Appl. Genet. − 2012. − **124**. − P. 189−206.

57. *Pshenichnikova T.A., Lapochkina I.F., Shchukina L.V.* The inheritance of morphological and biochemical traits introgressed into common wheat (*Triticum aestivum* L.) from *Aegilops speltoides* Tausch. // Genet. Res. Crop Evol. − 2007. − **54**. − P. 287−293.

58. *Liu Q., Ni Zh., Peng H. et al.* Molecular mapping of a dominant non-glaucousness gene from synthetic hexaploid wheat (*Triticum aestivum* L.) // Euphytica. − 2007. − **155**. − P. 71−78.

59. *Fu Sh., Sun Ch., Yang M. et al.* Genetic and epigenetic variations induced by wheat-rye 2R and 5R monosomic addition lines // PLoS One. − 2013. − **8**, № 1. − e54057.

60. *Tang Z., Fu Sh., Yan B. et al.* Unequal chromosome division and inter-genomic translocation occurred in somatic cells of wheat-rye allopolyploid // J. Plant Res. − 2012. − **125**. − P. 283−290.

61. *Pumphrey M., Bai J., Laudencia-Chingcuanco D. et al.* Nonadditive expression of homoeologous genes is established upon polyploidization in hexaploid wheat // Genetics. − 2009. − **181**. − P. 1147−1157.