АГРОНОМІЯ

The degree of genetic stability of amphidiploids from *Triticinae* tribe

Vdovychenko Zh.¹ [ID] **, Cichoński Ja.²** [ID] **, Shubenko L.³** [ID] **, Chrzanowski G.¹** [ID]

¹ *Institute of Biology, University of Rzeszów, Poland*
² *Doctoral School of the University of Rzeszów, University of Rzeszów, Poland*
³ *Bila Tserkva National Agrarian University, Ukraine*

✉➜ E-mail: Chrzanowski G.gchrzanowski@ur.edu.pl

OPEN ACCESS

Modern cultivars of wheat have narrowed genetic diversity. Their further improvement requires the expansion and enrichment of the gene pool. One of the sources of valuable genes for adaptive traits is considered to be related wild species of wheat, the involvement of which is possible through remote hybridization. However, the wide application of this method is severely limited due to a certain level of biological incompatibility of the crossed species: there is often no normal chromosome pairing, which leads to low fertility and the transfer of foreign genes to the genetic background of cultivated species as whole chromosomes or large translocations. Also, amphidiploids and lines with foreign introgressions show a certain level of genetic instability, because they experience the so-called «genomic shock».

The purpose of this research was to study the «genomic shock» in amphidiploids from species of the tribe *Triticinae*, from the genus *Aegilops* and *Triticum*. The plants were analyzed by a set of morphological features and electrophoretic spectra of storage proteins (gliadins). A search was made for plants that would have deviations from the typical morphotype or electrophoretic profile of the corresponding amphidiploid. Such deviations could indicate the course of «genomic shock» caused by polyploidization. The study of this phenomenon is important to find methods of accelerating diploidization processes, restoration of genetic stability and normal fertility in amphidiploids.

The results of the study showed the stable expression of the morphological features in all studied amphidiploids. Examination of the electrophoretic spectra of gliadins revealed the presence of some grains, which were marked either by the presence of additional protein components or by the absence of certain components. It cannot be ruled out that the appearance of atypical grains among the offspring of the studied amphidiploids may be a consequence of the «genomic shock».

Key words: remote hybridization, genomic shock, gliadins, amphidiploids, *Triticinae*.

Problem statement and analysis of recent research. Genetic diversity among modern cultivated plants is somewhat limited. This is due to the fact that during domestication, all crops passed through the so-called genetic «bottleneck» [22, 48], including wheat [9]. The second «bottleneck» was modern breeding, which contributed to the rapid spread of a limited number of high-yielding varieties [48, 22, 45]. Further improvement of wheat varieties requires the search for new donors of useful trait genes [30, 31]. It has been shown that natural populations of wild ancestors or species related to cultivated plants have the greatest genetic diversity [38, 49, 54]. Remote hybridization can contribute to the expansion of the gene pool of agricultural crops [20, 34, 50] and also be used to obtain new synthetic types of cultivated plants, examples of which are triticale [26], joshta [6].

When crossing crops with their relatives, including wild species, the genes that control the

best features of adaptability to biotic and abiotic stresses and other useful characteristics are transferred (introgressed) to the genome of this culture. To date, a great variety of introgressive lines for wheat has been obtained by crossing with a wide range of wild and cultivated relatives [7, 18, 19, 31, 50]. Although it is not always known in detail which foreign genes are responsible for the acquired trait of interest, what is the volume of foreign genetic material and what is its localization in the genome of the newly created line [17, 31].

Despite long-standing and numerous attempts to partially introduce foreign genetic material into the genome of cultivated plants, in particular, into wheat, today there are very few commercial varieties created in this way. A positive example is the acquisition of wheat-rye translocations 1BL/1RS and 1AL/1RS, which are used in modern wheat varieties. Translocation of the short arm of rye chromosome 1R confers resistance to a complex of fungal diseases [23, 39], but at the same time reduces the quality of flour [29].

Another example of commercial use of introgressive varieties is the Chinese common wheat variety Xiaoyan 6, which was widely cultivated in the 1980s and 1990s. In Xiaoyan 6, at least two wheat chromosomes (2A and 7D) carry chromosome segments from *Thinopyrum ponticum*, with genes contributing to disease and environmental stress resistance, as well as good yield quality and stability [21].

The reason for insufficient use of the adaptive potential of wild relatives is that in remote hybrids, due to genetic and cytological differences, normal conjugation of chromosomes in meiosis often does not occur and, accordingly, the exchange of chromosome sections, crossing over. The gene of interest is transferred to the background of the cultivated species, as a rule, either as part of a whole foreign chromosome, or by a large translocation [46, 51–53, 56]. Therefore, along with valuable genes for resistance to biotic and abiotic factors, a whole block of foreign genes is transferred, which significantly worsens the quality and yield characteristics of the crop [7, 31]. In addition, the presence of introgression due to the «incompatibility» of the genetic material of the donor and the recipient variety can lead to cytological instability and, as a result, to a significant decrease in plant fertility, which means a significant decrease in yield [37, 53]. Bringing a line with foreign-substituted chromosomes or parts of chromosomes to the quality standards of the crop is a rather complex breeding task. A widely known method to facilitate homeologous recombination, that is to induce exchange between donor and recipient chromosomes, is the use

of a *CSph1b* mutant [6]. However, successful introgression is still largely contingent.

In contrast to the task of transfer of only individual genes to the background of a cultured species, another direction of remote hybridization is the production of new synthetic species based on amphidiploids. An example of obtaining a new commercially successful synthetic species is triticale, which combines almost complete genomes of the parental forms of wheat and rye [26].

Cytological and molecular studies indicate that many cultivated plants arose as a result of spontaneous crossing between related species and selection carried out by man at the time of the birth of agriculture. That is probably what happened with durum and common wheat, oat, rapeseed, etc. Advances in sequencing technologies and bioinformatic study of plant genomes allowed us to assert that the phenomenon of polyploidization is a natural process and took place at the initial stage of the evolution of angiosperms [5, 10]. In addition, some taxa of angiosperms underwent additional polyploidization events in their development. That is, it can be argued that polyploidization served as one of the factors of speciation. The concept of a «paleopolyploid» appeared, a species that arose as a result of an ancient polyploidization event [8]. A paleopolyploid has a diploid-type chromosome conjugation, but the doubling of chromosomes that occurred during the formation of this species can still be traced through careful analysis of the nucleotide sequence of its genome. It is shown that, among cultivated plants, paleopolyploids are, in particular, corn, rice, and soybean [13, 35, 42]. Since this process took place repeatedly in nature and led to the appearance of new highly fertile plant species, it can be argued that remote hybridization is a technique that reproduces natural processes, and therefore, regardless of the difficulties that arise, one can hope for its success application for selection of new synthetic species closely related to cultivated plants. In view of this, in recent years there has been increased interest in the study of genetic processes that occur when genomes from different sources are combined in one organism.

Studies of amphidiploids demonstrate that when the genomes of two different species are combined in one nucleus, a so-called genomic shock occurs. The term for this phenomenon was proposed by Barbara McClintock (1984). Apparently, genomic shock is a programmed chromatin restructuring necessary to restore diploid chromosome conjugation and balance the genetic set. The consequences of genomic shock can be observed at different levels of organization of genetic material. Cytological instability is observed at the chromosomal level: aneuploidy occurs in the offspring of amphidiploids

and introgressive lines (lines with foreign genetic material), whole chromosomes can be eliminated [16, 34] or their parts [14], translocations occur [11, 46], specific sequences are eliminated [47]. At the level of nucleotide organization, the activation of transposons, changes in DNA methylation, histone modifications (epigenetic changes) are observed [1]. The appearance of new DNA sequences or protein components that were not characteristic of any of the parent organisms is often observed [2, 3, 15, 32, 33, 55]. The combination of two genomes does not lead to a simple summation of genes, where each of the genomes contributes equally to the functioning of the new organism. Most of such genomes are characterized by a decrease in the amount of chromatin compared to the expected. Various methods of interaction between homeologous genes are observed: the silencing of one of the homeologs or significant asymmetry in the expression of homeologs, their acquisition of different functions [2].

Revealing the causes of destabilization of the genomes of amphidiploids and introgressive lines and further molecular genetic mechanisms of their stabilization will be of both practical and theoretical interest. In practical breeding, this will allow for the acquisition of new synthetic, cytologically stable, fertile plant cultures. The theoretical value lies in understanding the mechanisms of the functioning of the plant genome as a whole.

Molecular-genetic processes occurring in the genome of amphidiploids and introgressive lines as a result of genomic shock can be monitored by genetic markers: morphological, biochemical, and molecular.

The aim of the research. This paper is devoted to the study of the expression stability of the morphological features and the electrophoretic spectra of gliadins in amphidiploids from species of the *Triticinae* tribe.

Materials and methods of research. Laboratory and field work was carried out in the Laboratory of storage proteins and at the experimental field of the Bila Tserkva National Agrarian University, respectively, in 2021–2022 years.

Amphidiploids obtained from crossing species of the *Triticinae* tribe served as plant material (Table 1). Seeds of plant samples were kindly provided by the Bank of Genetic Resources (Kharkiv).

The genetic stability of amphidiploids was studied by evaluating morphological features of plants and electrophoretic profile of storage proteins, gliadins.

Evaluation of morphological features
At the stage of flowering, the plants were evaluated by morphological features such as the presence of a waxy coat, the pubescence of the leaf, and the color of the auricles. After ripening, the plants were evaluated on the characteristics of awnedness, color, and hardness of the glumes, the presence of an indentation at the base of the glume (the middle spikelets of the main spike were evaluated), the shape, and density of the spike. The gradations of the traits are given in table 2. Each amphidiploid was characterized by the stability of the expression of morphological features by evaluating about 50 plants, each of which in turn formed several stems.

Electrophoresis of gliadins
Extraction and electrophoresis of gliadins in a polyacrylamide gel was performed according to the modified Brzezinski method (Антонюк et al., 1994). For each amphidiploid, 55 grains were evaluated, except for the sample *T. durum – Ae. squarrosa* 30, which was not examined for the profile of gliadins.

Table 1 – **Amphidiploids used in the study**

No	Origin of the sample	Notes	Number of chromosomes
1	*Ae. squarrosa – T. boeoticum*		28
2	*Ae. squarrosa – T. urartu*		28
3	*T. durum – Ae. squarrosa*	Sample 24	42
4	*T. durum – Ae. squarrosa*	Sample 30, without wax coating	42
5	*T. durum – Ae. squarrosa*	Sample 1	42
6	Tetra-Aurora* – *Ae. mutica*	Aurotica	42
7	*T. durum – Ae. comosa*	Miosa	42

* – AABB genomes of common wheat variety Aurora.

Table 2 – **Gradations of morphological traits by which plants were evaluated**

Trait	Gradation
Wax coating	present / absent
Pubescence of the upper surface of the leaf	present / absent
The colour of the auricles	light green / purple
Awnedness	awnless / awned / semi-awned / with awn-like processes
The shape and density of the spike	spindle-shaped / speltoid / loose
The colour of glumes	white / yellow / red / light brown / dark brown
Glume hardness	soft / hard / very hard
The indentation of the spikelet base	present / absent / weakly expressed

Research results and discussion. *Evaluation by morphological traits.*

The spike morphology of the investigated amphidiploids is presented in Figures 1–3.

The characteristics of amphidiploids according to gradations of morphological features are presented in Table 3. For comparison, the table also shows the characteristic gradations of common wheat. Each of the investigated amphidiploids showed its own characteristics, on the basis of which it was possible to control the uniformity of the plants of the sample. Amphidiploids showed stability in the expression of morphological traits: the plants of all samples were uniform.

Most of the plants in the sample *T. durum – Ae. squarrosa* 24 were without a waxy coating and with purple auricles, but among them there were also plants that had two traits changed at the same time, they were with a waxy coating and light green auricles. According to the characteristics of the spike morphology (awnedness, shape), all plants in the sample were similar. Further comparison of these morphologically different plants with typical plants according to the electrophoretic profiles of gliadins revealed a significant difference in the composition of protein components (Fig. 4–8). Given this, we can make an assumption that it is possible that these two groups of plants in the sample *T. durum – Ae. squarrosa* 24 have a suchlike origin, as evidenced by the similar morphology of the ear. But the significant differences in the set of storage proteins allow us to say that they do not come from the same line. This explanation is more likely than the manifestation of genetic instability in this amphidiploid. Therefore, in the future, it makes sense to conduct research on these two groups of plants separately. A well-founded conclusion about the nature of genetic processes in the genomes of the studied amphidiploids can be made after further multiple screening of the plant material and observations.

Evaluation of electrophoretic profiles of gliadins

For each of the amphidiploids, 55 grains were evaluated, except for the sample *T. durum – Ae. squarrosa* 30, which was not examined for the spectrum of gliadins. Examples of electrophoregrams are presented in Figures 4–8.

For the amphidiploid *Ae. squarrosa – T. boeoticum* and *Ae. squarrosa – T. urartu*, one grain was found for each, showing additional components (Fig. 4).

For the amphidiploid *T. durum – Ae. squarrosa* 24, a significant difference in the protein profiles was found between two groups of plants (Fig. 5), which also differed due to the presence of a waxy coating. Our assumptions regarding this circumstance were expressed before.

For the amphidiploid *T. durum – Ae. squarrosa* 1, two groups of plants were identified, which differed from each other by the block of polypeptides in the ω-zone of the spectrum (Fig. 6).

For Aurotica, two grains with a missing block of components were found in the γ and β spectrum zones (not shown). For Miosa, two grains with missing components in the ω- and β-zones of the spectrum were found (Fig. 7).

Therefore, as a result of the study of amphidiploids according to the electrophoretic spectrum of gliadins, a small number of grains with atypical spectra were found (Table 4). It cannot be ruled out that the appearance of such plants is a consequence of genetic processes in the genomes of these samples, launched by the polyploidization event, that is, due to a genomic shock.

240

Fig. 1. **Morphology of the spike of the samples *Ae. squarrosa – T. boeoticum* (A),**
***Ae. squarrosa – T. urartu* (B).**

Fig. 2. **Morphology of the spike of the samples *T. durum – Ae. squarrosa* 24 (A),**
***T. durum – Ae. squarrosa* 30 (B), *T. durum – Ae. squarrosa* 1 (C).**

Fig. 3. **Morphology of the spike of the samples Tetra-Aurora –**
***Ae. mutica* (Aurotica)** (A), ***T. durum – Ae. comosa* (Miosa)** (B).

Table 3 – **Evaluation of amphidiploids by morphological traits**

No	Origin of the sample	Wax coating	Pubescence of the upper surface of the leaf	The colour of the auricles	Awned-ness	The shape and density of the spike	The colour of glumes	Glume hard-ness	The indentation of the spike-let base
1	*Ae. squarrosa – T. boeoticum*	absent	present	light green	semi-awned	loose	dark brown	very hard	absent
2	*Ae. squarrosa – T. urartu*	absent	present	light green	semi-awned	loose	dark brown	very hard	absent
3	*T.durum – Ae. squarrosa* (24)	absent	absent	purple	awned	spin-dle-shaped	dark brown	hard	weakly expressed
4	*T. durum – Ae. squarrosa* (30)	absent	absent	light green	awned	spin-dle-shaped	yellow	hard	weakly expressed
5	*T. durum – Ae. squarrosa* (1)	present	absent	light green	semi-awned	spin-dle-shaped	white	hard	weakly expressed
6	Tetra-Aurora *Ae. mutica* (Aurotica)	absent	absent	light green	awnless	speltoid	white	hard	absent
7	*T. durum – Ae. comosa* (Miosa)	absent	absent	purple	with awn-like processes	speltoid	white	hard	absent
8	*T. aestivum* (common wheat)	present	absent	light green	different grada-tions	spin-dle-shaped	white / yellow / red	soft	present

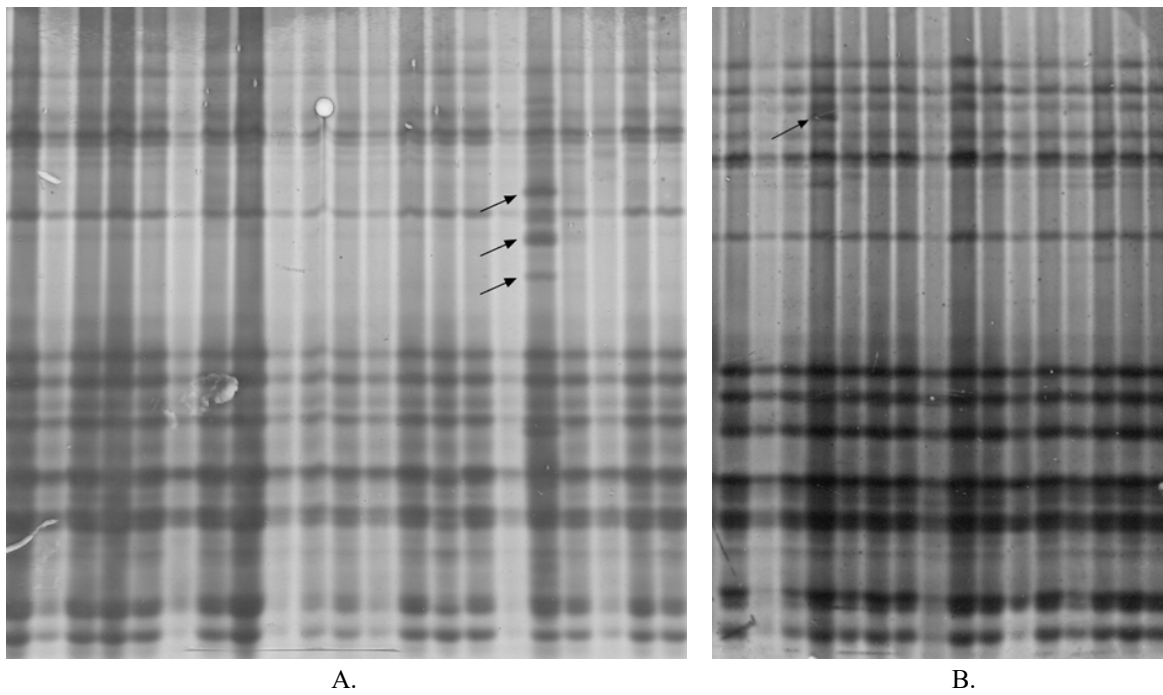Fig. 4. **Spectrum of gliadin of the samples *Ae. squarrosa – T. boeoticum* (A),
Ae. squarrosa – T. urartu (B). Arrows point to additional components.**
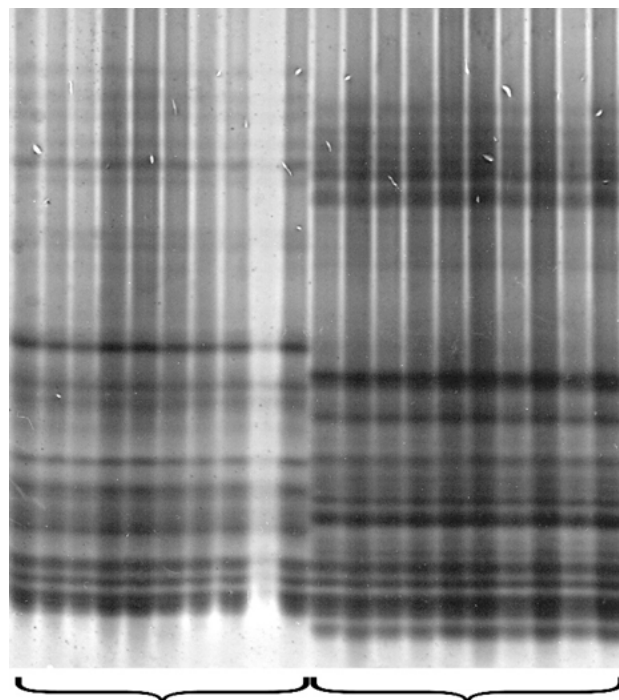

Fig. 5. **Profile of gliadins of the sample *T. durum –
Ae. squarrosa* 24. The brackets indicate two groups
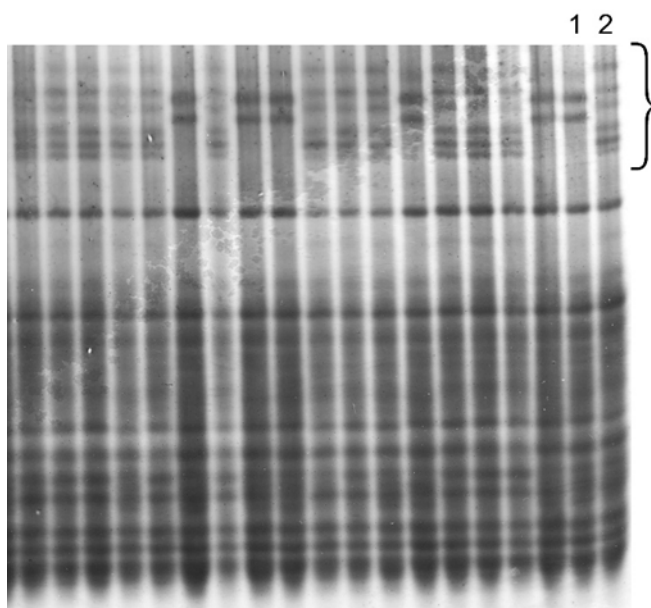of plants that differ in electrophoretic profiles and
morphological traits.**

Fig. 6. **Spectrum of gliadins of the sample *T. durum –
Ae. squarrosa* 1. A bracket indicates a zone with two
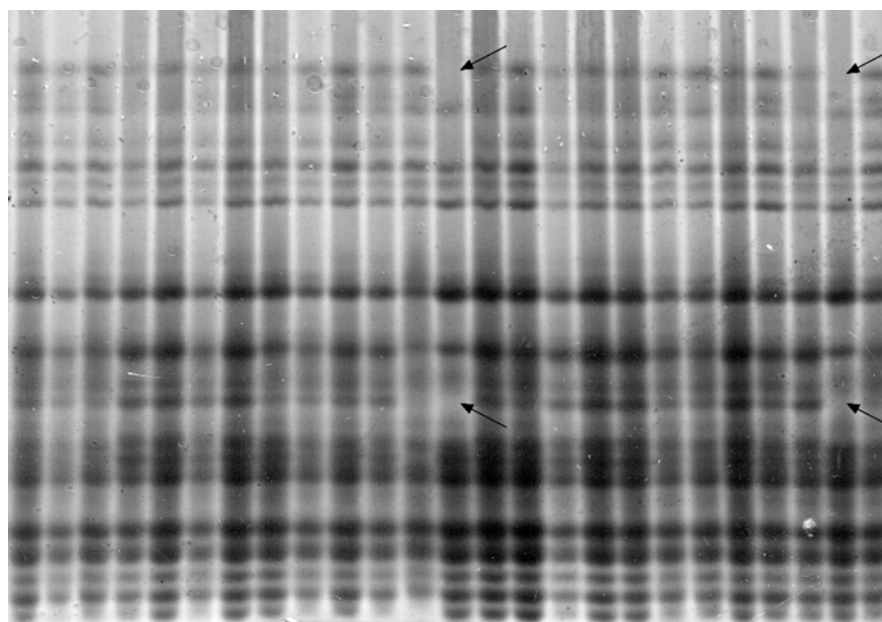types of protein blocks. Samples with each block are
marked with numbers 1 or 2.**

Fig. 7. **Spectrum of gliadins of the sample *T. durum – Ae. comosa* (Miosa).
Arrows indicate missing components.**

Table 4 – **The examination of the amphidiploids according to the electrophoretic spectra of gliadins and morphological traits**

No	Sample	The number of atypical grains *	Type of deviation	Morphological traits
1	*Ae. squarrosa – T. boeoticum*	1	3 additional components in the γ-zone	Uniform
2	*Ae. squarrosa – T. urartu*	1	1 additional component in the ω-zone	Uniform
3	*T. durum – Ae. squarrosa* 24	2 groups of spectra	Mechanical contamination	2 groups of phenotypes
4	*T. durum – Ae. squarrosa* 30	–	–	Uniform
5	*T. durum – Ae. squarrosa* 1	2 groups of spectra	2 types of blocks in the ω-zone	Uniform
6	Tetra-Aurora – *Ae. mutica* (Aurotica)	1	The block of components in the area of γ and β-zones is missing	Uniform
7	*T. durum – Ae. comosa* (Miosa)	2	1 component in the ω-zone and 1 component in the β-zone are missing	Uniform

* the sample volume is 55 grains.

Application of morphological markers

Morphological markers allow visual assessment of plants without additional expensive laboratory procedures. The disadvantage of working with morphological features is that only those controlled by one or a small number of genes can be used as markers; the trait should appear regardless of environmental conditions and with a clear gradation. The number of such features is significantly limited and is not sufficient for dense marking of the entire plant genome; therefore, there is a need to use additional biochemical and molecular markers. Nevertheless, the study of available morphological markers allows us to draw the first conclusions regarding the stability of the genome. For many morphological features of soft wheat, their genetic control has been established and the chromosomal location of the genes encoding them is known. The phenomenon of synteny, i.e. the preservation of the order of genes in chromosomes in closely related species allows us to use this information in the study of amphidiploids from closely related species.

The dark color of the mature glume is controlled by the *Rg2* gene, located on chromosome 1D [51]. Orthologous genes are located on the first chromosome of other wheat genomes [25]. The development of a waxy coating on a plant is caused by W genes located on the short arms of chromosomes 2B and 2D [24]. The *W2* inhibitor gene, located on the 2DS shoulder, is responsible for the absence of a waxy coating. In modern varieties of soft wheat, this gene is represented by the recessive allele *w2*, so the plants are covered with wax.

Common wheat has a spindle-shaped spike. In amphidiploids, the appearance of a loose ear is caused by a gene contained in the chromosome of the sixth homeologous group [28]. The genes responsible for the hardness of the glume and the presence of an indentation at its base (a characteristic of common wheat) are located on chromosome 2D [51].

Anthocyanin coloration is caused by genes located on chromosomes of the 7th homeologous group. They cause purple coloration of coleoptile, straw, anthers [25]. We assume that the purple auricles on the leaf are also the pleiotropic action of this gene. An example of the successful use of morphological markers to assess the stability of the genomes of amphidiploids and introgressive lines is the paper by Ternovskaya T., Zhirov E. (1993). The evaluation of Miosa and MIT amphidiploids based on the presence of a wax coating showed that at the time of creation, these amphidiploids were without wax, but after a certain number of generations this feature appeared. These changes in the genome of the studied plant samples turned out to be irreversible and had hereditary nature.

The amphidiploids we studied had a number of morphological features that allowed us to clearly distinguish them, and also distinguished them from the common wheat morphotype. When examining plants from one generation of each amphidiploid, no diversity in morphology was found. It can be assumed that the most active adaptation processes of the amphidiploid subgenomes occur in the first generations after polyploidization. Also, more information can be obtained by observing an amphidiploid in several generations. To activate genomic shock, we crossed all amphidiploids with common wheat to obtain new introgressive lines. We expect that the combination of genetic material of various parental components will cause new processes of its reconstruction and mutual adaptation. This plant material can be used in further studies of the phenomenon of genomic shock.

Application of biochemical markers

Biochemical markers for tracking genetic changes in amphidiploids and introgressive lines were used in several works by the team of authors [3, 32, 33]. Grain gliadins, α- and β-amylase isoenzymes were used in these works. The results showed the appearance of new components of the electrophoretic spectra that were not characteristic of the parent plants. The authors suggested that such changes could be associated with the activation of transposons that are contained in the intergenic space of gliadins, as well as due to «slipping» of DNA polymerase during replication, since gliadin genes are rich in microsatellite repeats, in particular the SAA repeat, or due to uneven crossing over because of the present of microsatellite repeats and repeats in transposons.

Six loci are known, located distally on the short arms of chromosomes of the 1st and 6th homeologous groups, encoding gliadins: *Gli-A1, Gli-B1, Gli-D1, Gli-A2, Gli-B2,* and *Gli-*D2 [36, 43]. Each of the gliadin genes encodes several polypeptides that are inherited as a single Mendelian trait. On the electrophoretic spectrum, the gliadin gene forms a block of components [44]. Gliadins are characterized by multiple polymorphism, so it is convenient to use them as markers for distinguishing not only *Triticinae* species, but also for distinguishing individual varieties. Catalogues of wheat gliadin variants have been created [27].

According to the growth of the electrophoretic mobility of the gliadin components, the spectrum is divided into 4 zones: ω, γ, β and α. The first two zones are mainly controlled by genes located on the short arms of chromosomes of the first group, and the other two by genes located on the short

arms of the chromosomes of the sixth group [41], although sometimes the products of the same gene can be detected in different zones [12].

The differences in the electrophoretic spectra of amphidiploids studied by us concerned the ω, γ, β zones. We observed the lack of some components in some cases, and the appearance of new bands in the spectra in other cases. The presence of two groups of plants with different blocks of components in *T. durum – Ae. squarrosa* (1) most likely indicates a single mutational event as a result of genomic shock and subsequent preservation and reproduction from seeds of both groups in this amphidiploid population. The same can be assumed for the appearance of two grains with deviations of the same nature in Miosa samples.

Although in most cases the changes in the spectra we observed involved more than one component at the same time, it is most likely that the mutational event occurred at only one locus in each case. However, we cannot exclude the fact that such spectra are due to mutations in more than one locus.

Use of DNA markers

The REMAP (retrotransposon-microsatellite amplification polymorphism) method can serve as one of the approaches to the study of rearrangements of nucleotide sequences that may occur as a result of the activity of transposons. This method consists in conducting a polymerase chain reaction with primers that have the following features: one of the primers is complementary to the sequence in the LTR-transposon, the second primer is complementary to the microsatellite sequence and also has an anchor nucleotide at the end. This method turned out to be effective, since retrotransposons are often associated with microsatellites in the cereal genome [40]. It allows detection of the movement of the transposon relative to the microsatellite when evaluating changes in the length of DNA fragments formed during amplification. In work [2], the Sukkula family retrotransposon sequence was used as a donor of a conservative region of the retrotransposon, since it is considered one of the families with the highest activity of translocations throughout the genome. A total of 49 generations in 19 introgressive lines of Aurodes derivatives were analysed. The vast majority of the studied lines had differences in the REMAP spectrum either in general for the line, in individual generations, or in individual grains. The authors concluded about the significant mobility of retrotransposons in the genome of introgressive lines of wheat. Thus, the use of DNA markers, in particular the

study of transposons, indicates active processes of genome rearrangement due to genomic shock in introgressive lines of wheat.

Accumulating evidence that remote hybridization itself is a factor in the growth of genetic variability in offspring compared to parental genotypes increases interest in it and actualizes its research. As the genome of the plant destabilizes and begins to actively reconstruct, this phenomenon in itself can be used to create a new variety of offspring for the selection process.

Our further efforts will be focused on creating suitable plant material to study the phenomenon of "genomic shock". It is necessary to isolate plants with atypical morphological, biochemical, and molecular markers and further study the stability of the expression of acquired changes in subsequent generations.

Conclusions. Amphidiploids involved in the study, based on the results of observation for one year, showed a stable expression of morphological features. The study of gliadin profiles of amphidiploids demonstrated the appearance of individual cases of atypical grains. In their profile, either new components appeared, or some components disappeared. In one of the cases, an entire block of components was missing. It cannot be ruled out that the appearance of atypical grains may be a consequence of «genomic shock».

REFERENCES

1. Antonyuk, M., Shpylchyn, V., Martynenko, V., Ternovska, T. (2022). Significance of introgression hybridization for extension of genetic variability in recipient genome, NRPBE. no. 5, pp. 3–13. DOI: 10.18523/2617-4529.2022.5.3-13

2. Antonyuk, M.Z. (2019). Introgression as inductor of wheat Triticum aestivum L. genome variability: dissertation. Kyiv, Ukraine, Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine. 547 p.

3. Antonyuk, M.Z., Shpylchyn, V.V., Ternovska, T.K. (2013). Permanent genetic variability in the introgressive lines and amphidiploids of Triticeae, Cytol. Genet. no. 47, pp. 242–251. DOI: 10.3103/S0095452713040026

4. Antonyuk, M.Z., Ternovskaya, T.K., Sozinov, A.A. (1994). Identification of the blocks of electrophoretic components of storage proteins encoded by the genes of three Aegilops species, Fiziol. Biokhim. Kul'turnykh Rasteniy. no. 5, pp. 474–481.

5. Aversano, R., Ercolano, M.R., Caruso, I., Fasano, C., Rosellini, D., Carputo D. (2012). Molecular Tools for Exploring Polyploid Genomes in Plants, IJMS. no. 13, pp. 10316–10335. DOI: 10.3390/ijms130810316

6. Bauer, A. (1986). New results of breeding Ribes nidigrolaria: amphidiploid species hybrids between blackcurrant and gooseberry, Acta Hortic. pp. 107–110.

7. Burt, C., Nicholson, P. (2011). Exploiting co-linearity among grass species to map the Aegilops ventricosa-derived Pch1 eyespot resistance in wheat and establish its relationship to Pch2, Theor Appl Genet. no. 123, pp. 1387–1400. DOI: 10.1007/s00122-011-1674-9

8. Cannon, S. (2008). Legume Comparative Genomics, in: G. Stacey (Ed.), Genetics and Genomics of Soybean, Springer New York. New York, NY, pp. 35–54. DOI: 10.1007/978-0-387-72299-3_3

9. Charmet, G. (2011). Wheat domestication: Lessons for the future, Comptes Rendus Biologies. no. 334, pp. 212–220. DOI: 10.1016/j.crvi.2010.12.013

10. Debodt, S., Maere, S., Vandepeer, Y. (2005). Genome duplication and the origin of angiosperms, Trends in Ecology & Evolution. no. 20, pp. 591–597. DOI: 10.1016/j.tree.2005.07.008

11. Fu, S., Tang, Z., Ren, Z. (2010). Inter- and intra-genomic transfer of small chromosomal segments in wheat-rye allopolyploids. J Plant Res. no. 123, pp. 97–103. DOI: 10.1007/s10265-009-0264-2

12. Gao, S., Gu, Y.Q., Wu, J., Coleman-Derr, D., Huo, N., Crossman, C., Jia, J., Zuo, Q., Ren, Z., Anderson, O.D., Kong, X. (2007). Rapid evolution and complex structural organization in genomic regions harboring multiple prolamin genes in the polyploid wheat genome. Plant Mol Biol. no. 65, pp. 189–203. DOI: 10.1007/s11103-007-9208-1

13. Gaut, B.S. (2001). Patterns of Chromosomal Duplication in Maize and Their Implications for Comparative Maps of the Grasses. Genome Res. no. 11, pp. 55–66. DOI: 10.1101/gr.160601

14. Gernand, D., Rutten, T., Varshney, A., Rubtsova, M., Prodanovic, S., Brüß, C., Kumlehn, J., Matzk, F., Houben, A. (2005). Uniparental Chromosome Elimination at Mitosis and Interphase in Wheat and Pearl Millet Crosses Involves Micronucleus Formation, Progressive Heterochromatinization, and DNA Fragmentation, Plant Cell. no. 17, pp. 2431–2438. DOI: 10.1105/tpc.105.034249

15. Han, F.P., Fedak, G., Ouellet, T., Liu, B. (2003). Rapid genomic changes in interspecific and intergeneric hybrids and allopolyploids of Triticeae, Genome. no. 46, pp. 716–723. DOI: 10.1139/g03-049

16. Ishii, T., Ueda, T., Tanaka, H., Tsujimoto, H. (2010). Chromosome elimination by wide hybridization between Triticeae or oat plant and pearl millet: pearl millet chromosome dynamics in hybrid embryo cells, Chromosome Res. no. 18, pp. 821–831. DOI: 10.1007/s10577-010-9158-3

17. Johansson, E., Henriksson, T., Prieto-Linde, M.L., Andersson, S., Ashraf, R., Rahmatov, M. (2020). Diverse Wheat-Alien Introgression Lines as a Basis for Durable Resistance and Quality Characteristics in Bread Wheat, Front. Plant Sci. no. 11, 1067 p. DOI: 10.3389/fpls.2020.01067

18. King, I.P., Forster, B.P., Law, C.C., Cant, K.A., Orford, S.E., Gorham, J., Reader, S., Miller, T.E. (1997).

Introgression of salt-tolerance genes from Thinopyrum bessarabicum into wheat, New Phytol. no. 137, pp. 75–81. DOI: 10.1046/j.1469-8137.1997.00828.x

19. Kozub, N.A., Sozinov, I.O., Bidnyk, H.Ya., Demianova, N.O., Sozinova, O.I., Yanse, L.A., Karelov, A.V., Blume, Ya.B. (2021). Grain quality indices in common wheat lines with introgressions of chromosome 1U from Aegilops biuncialis Vis., Fakt. Eksp. Evol. Org. no. 29, pp. 87–91. DOI: 10.7124/FEEO.v29.1412

20. Kuzmanović, L., Rossini, F., Ruggeri, R., Pagnotta, M.A., Ceoloni, C. (2020). Engineered Durum Wheat Germplasm with Multiple Alien Introgressions: Agronomic and Quality Performance, Agronomy. no. 10, 486 p. DOI: 10.3390/agronomy10040486

21. Li, Z., Li, B., Tong, Y. (2008). The contribution of distant hybridization with decaploid Agropyron elongatum to wheat improvement in China. Journal of Genetics and Genomics. no. 35, pp. 451–456. DOI: 10.1016/S1673-8527(08)60062-4

22. Louwaars, N.P. (2018). Plant breeding and diversity: A troubled relationship? Euphytica. no. 214, 114 p. DOI: 10.1007/s10681-018-2192-5

23. Mago, R., Spielmeyer, W., Lawrence, G., Lagudah, E., Ellis, J., Pryor, A. (2002). Identification and mapping of molecular markers linked to rust resistance genes located on chromosome 1RS of rye using wheat-rye translocation lines. Theor Appl Genet. no. 104, pp. 1317–1324. DOI: 10.1007/s00122-002-0879-3

24. McClintock, B. (1984). The Significance of Responses of the Genome to Challenge. Science. no. 226, pp. 792–801. DOI: 10.1126/science.15739260

25. McIntosh, R.A., Yamazaki, Y., Dubcovsky, J., Rogers, J., Morris, C., Somers D.J. (2014). Catalogue of gene symbols for wheat. Available at: https://shigen.nig.ac.jp/wheat/komugi/genes/download.jsp

26. Mergoum, M., Singh, P.K., Peña, R.J., Lozano-del Río, A.J., Cooper, K.V., Salmon, D.F., Gómez Macpherson, H. (2009). Triticale: A «New» Crop with Old Challenges, in: M.J. Carena (Ed.), Cereals, Springer US. New York, NY, pp. 267–287. DOI: 10.1007/978-0-387-72297-9_9

27. Metakovsky, E., Melnik, V., Rodriguez-Quijano, M., Upelniek, V., Carrillo, J.M. (2018). A catalog of gliadin alleles: Polymorphism of 20th-century common wheat germplasm. The Crop Journal. no. 6, pp. 628–641. DOI: 10.1016/j.cj.2018.02.003.

28. Miller, T.E., Reader, S.M. (1987). A guide to the homoeology of chromosomes within the Triticeae. Theoret. Appl. Genetics. no. 74, pp. 214–217. DOI: 10.1007/BF00289971

29. Morgun, B.V. (2016). State and perspectives of wheat-rye translocations use in winter wheat breeding. Fiziol. Rast. Genet. no. 48, pp. 324–343. DOI: 10.15407/frg2016.04.324

30. Morgun, V.V., Topchii, T.V. (2018). The importance of resistant varieties of winter wheat, the study of sources and donors of resistance to pests and main pathogen. Fiziol. Rast. Genet. no. 50, pp. 218–240. DOI: 10.15407/frg2018.03.218

31. Motsnyi, I.I., Molodchenkova, O.O., Nargan, T.P., Nakonechnyy, M.Yu., Mishchenko, I.A., Lyfenko, S.Ph., Smertenko, A.P., Mishchenko, L.T. (2022). Impact of Alien Genes on Disease Resistance, Drought Tolerance, and Agronomic Traits in Winter Wheat Commercial Varieties. TOASJ. no. 16, e187433152111260. DOI: 10.2174/18743315-v16-e2111260

32. Mykhailyk, S.Y., Antonyuk, M.Z., Ternovska, T.K. (2011). Genetic variability of common wheat introgressive lines for the Gli genes. NaUKMA Research Papers. no. 119, pp. 8–14. Available at: https://ekmair.ukma.edu.ua/handle/123456789/3638

33. Mykhailyk, S.Y., Antonyuk, M.Z., Ternovska, T.K. (2014) Possible molecular mechanisms of variability in gliadin genes in the wheat introgressive lines. Factors in Experimental Evolution of Organisms. no. 14, pp. 62–66. Available at: http://utgis.org.ua/journals/index.php/Faktory/article/view/236

34. Nemeth, C., Yang, C., Kasprzak, P., Hubbart, S., Scholefield, D., Mehra, S., Skipper, E., King, I., King, J. (2015). Generation of amphidiploids from hybrids of wheat and related species from the genera Aegilops, Secale, Thinopyrum, and Triticum as a source of genetic variation for wheat improvement. Genome. no. 58, pp. 71–79. DOI: 10.1139/gen-2015-0002

35. Paterson, A.H., Bowers, J.E., Chapman, B.A. (2004). Ancient polyploidization predating divergence of the cereals, and its consequences for comparative genomics. Proc. Natl. Acad. Sci. U.S.A. no. 101, pp. 9903–9908. DOI: 10.1073/pnas.0307901101

36. Payne, P.I., Holt, L.M., Lawrence, G.J., Law, C.N. (1982). The genetics of gliadin and glutenin, the major storage proteins of the wheat endosperm. Plant Food Hum Nutr. no. 31, pp. 229–241. DOI: 10.1007/BF01108632

37. Plyhun, V., Iefimenko, T., Antonyuk, M., Ternovska, T. (2020). Cytological stability of wheat amphidiploids and cultivars in meiosis I, NaUKMA Research Papers. Biology and Ecology. no. 3, pp. 3–13. DOI: 10.18523/2617-4529.2020.3.3-13

38. Pour-Aboughadareh, A., Kianersi, F., Poczai, P., Moradkhani, H. (2021). Potential of Wild Relatives of Wheat: Ideal Genetic Resources for Future Breeding Programs. Agronomy. no. 11, 1656 p. DOI: 10.3390/agronomy11081656

39. Rabinovich, S.V. (1998) Importance of wheat-rye translocations for breeding modern cultivar of Triticum aestivum L. Euphytica. no. 100, pp. 323–340. DOI: 10.1023/A:1018361819215

40. Ramsay, L., Macaulay, M., Cardle, L., Morgante, M., Ivanissevich, S., Maestri, E., Powell, W., Waugh, R. (1999). Intimate association of microsatellite repeats with retrotransposons and other dispersed

repetitive elements in barley, Plant J. no. 17, pp. 415–425. DOI: 10.1046/j.1365-313X.1999.00392.x

41. Ruiz, M., Aguiriano, E., Fité, R., Carrillo, J.M. (2007). Combined use of gliadins and SSRs to analyse the genetic variability of the Spanish collection of cultivated diploid wheat (Triticum monococcum L. ssp. monococcum). Genet Resour Crop Evol. no. 54, pp. 1849–1860. DOI: 10.1007/s10722-007-9208-7

42. Schlueter, J.A., Lin, J.-Y., Schlueter, S.D., Vasylenko-Sanders, I.F., Deshpande, S., Yi, J., O'Bleness, M., Roe, B.A., Nelson, R.T., Scheffler, B.E., Jackson, S.A., Shoemaker, R.C. (2007). Gene duplication and paleopolyploidy in soybean and the implications for whole genome sequencing. BMC Genomics. no. 8, 330 p. DOI: 10.1186/1471-2164-8-330

43. Singh, N.K., Shepherd, K.W. (1988). Linkage mapping of genes controlling endosperm storage proteins in wheat: 1. Genes on the short arms of group 1 chromosomes. Theoret. Appl. Genetics. no. 75, pp. 628–641. DOI: 10.1007/BF00289132

44. Sozinov, A.A., Poperelya, F.A. (1980). Genetic Classification of Prolamins and Its Use for Plant Breeding. Ann. Technol. Agric. no. 28, pp. 229–245.

45. Sthapit, S.R., Marlowe, K., Covarrubias, D.C., Ruff, T.M., Eagle, J.D., McGinty, E.M, Hooker, M.A., Duong, N.B., Skinner, D.Z., See, D.R. (2020). Genetic diversity in historical and modern wheat varieties of the U.S. Pacific Northwest. Crop Sci. no. 60, pp. 3175–3190. DOI: 10.1002/csc2.20299

46. Tanaka, H., Nabeuchi, C., Kurogaki, M., Garg, M., Saito, M., Ishikawa, G., Nakamura, T., Tsujimoto, H. (2017). A novel compensating wheat – Thinopyrum elongatum Robertsonian translocation line with a positive effect on flour quality. Breed. Sci. no. 67, pp. 509–517. DOI: 10.1270/jsbbs.17058

47. Tang, Z., Wu, M., Zhang, H., Yan, B., Tan, F., Zhang, H., Fu, S., Ren, Z. (2012). Loss of Parental Coding Sequences in an Early Generation of Wheat-Rye Allopolyploid. International Journal of Plant Sciences. no. 173, pp. 1–6. DOI: 10.1086/662655

48. Tanksley, S.D., McCouch, S.R. (1997). Seed Banks and Molecular Maps: Unlocking Genetic Potential from the Wild. Science. no. 277, pp. 1063–1066. DOI: 10.1126/science.277.5329.1063

49. Tenaillon, M.I. (2004). Selection Versus Demography: A Multilocus Investigation of the Domestication Process in Maize. Molecular Biology and Evolution. no. 21, pp. 1214–1225. DOI: 10.1093/molbev/msh102

50. Ternovska, T.K., Iefimenko, T.S., Antonyuk, M.Z. (2022). Improvement of Wheat Genetic Resistance to Powdery Mildew Retrospects and Prospects. TOASJ. no. 17, e187433152210310. DOI: 10.2174/18743315-v16-e221026-2022-HT14-3623-1

51. Ternovskaya, T., Zhirov, E. (1993). The Genetic Control of Waxiness, Hairy Glume, and Mature Spike Color, Tsitol. Genet. no. 27, pp. 15–20.

52. Vdovichenko, Z.V., Zlatskaia, A.V., Ternovskaia, T.K. (2001). New morphological marker for chromosomes of the fourth homologous group of Triticinae. Tsitol Genet. no. 35, pp. 28–33.

53. Vdovychenko, Zh.V. (2004). Introgressions in the genome of common wheat (Triticum aestivum L.) as a factor affecting the results of its genetic analysis [dissertation] Ukraine, Kyiv, Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine. 182 p. Available at: http://www.disslib.org/introhresiyi-v-henomi-m-jakoyi-pshenytsi-jak-faktor-shcho-vplyvaye-na-rezultaty-yiyi.html

54. Xu, D.H., Gai, J.Y. (2003). Genetic diversity of wild and cultivated soybeans growing in China revealed by RAPD analysis. Plant Breeding. no. 122, pp. 503–506. DOI: 10.1046/j.0179-9541.2003.00911.x

55. Zhao, N., Xu, L., Zhu, B., Li, M., Zhang, H., Qi, B., Xu, C., Han, F., Liu B. (2011). Chromosomal and genome-wide molecular changes associated with initial stages of allohexaploidization in wheat can be transit and incidental. Genome. no. 54, pp. 692–699. DOI: 10.1139/g11-028

56. Zlatskaya, A., Antonyuk, M., Vdovychenko, Z., Ternovskaya, T. (1999). Seed acid phosphatase as a genetic marker for homoeologous group 4 chromosomes in goat grass and wheat. Tsitol Genet. no. 33, pp. 35–38.

Ступінь генетичної стабільності амфідиплоїдів триби *Triticinae*

Вдовиченко Ж.В., Чіхоньський Я., Шубенко Л.А., Хшановський Г.

Сучасні сорти пшениці мають обмежену генетичну різноманітність. Подальше їх вдосконалення потребує розширення і збагачення генофонду. Одним із джерел цінних генів адаптивних ознак вважаються споріднені дикі види пшениці, залучення яких можливе за віддаленої гібридизації. Однак широке застосування цього методу досить обмежене через певний рівень біологічної несумісності схрещуваних видів. Часто немає нормальної кон'югації хромосом у мейозі гібридів, що призводить до низької плодючості та перенесення чужинних генів на генетичне тло культивованих видів у вигляді як цілих хромосом, так і великих транслокацій. Амфідиплоїди та лінії з чужинними інтрогресіями виявляють певний рівень генетичної нестабільності, оскільки переживають так званий «геномний шок».

Метою цього дослідження було вивчення «геномного шоку» у амфідиплоїдів видів триби *Triticinae*, з роду *Aegilops* і *Triticum*. Рослини аналізували за набором морфологічних ознак та електрофоретичних спектрів запасних білків (гліадинів). Було проведено пошук рослин, які мали б відхилення від типового морфотипу або електрофоретичного профілю відповідного амфідиплоїду. Такі відхилення можуть свідчити про перебіг «геномного шоку», зумовленого поліплоїдизацією. Вивчен-

ня «геномного шоку» важливо для пошуку методів прискорення процесів диплоїдизації, відновлення генетичної стабільності та нормальної фертильності амфідиплоїдів.

Результати дослідження показали стабільну експресію морфологічних ознак у всіх досліджених амфідиплоїдів. Дослідження електрофоретичних спектрів гліадинів виявило кілька одиничних зернівок, які відзначалися або наявністю додаткових білкових компонентів, або відсутністю певних компонентів. Слід враховувати, що поява атипових зернівок серед нащадків досліджуваних амфідиплоїдів може бути наслідком «геномного шоку».

Ключові слова: віддалена гібридизація, геномний шок, гліадини, амфідиплоїди, *Triticinae.*

ORCID iD:
Vdovychenko Zh.   https://orcid.org/0000-0002-6070-5518
Cichoński Ja.   https://orcid.org/0000-0003-3053-4153
Shubenko L.   https://orcid.org/0000-0002-8938-9520
Chrzanowski G.   https://orcid.org/0000-0001-9836-1514