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MOLECULAR-GENETIC CHARACTERIZATION OF SEED STORAGE PROTEIN COMPOSITION OF PARTIAL WHEAT-WHEATGRASS HYBRIDS

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Abstract: Seed storage proteins were studied in the collection of winter partial wheat-wheatgrass hybrids. The genes encoding high molecular weight glutenins (HMWG) of wheatgrass origin ha\'e been detected in 23 lines of partial wheat-wheatgrass hybrids using PCR technique. The genes are expressed in at least 15 of 23 lines that have been shown by protein electrophoresis. The wheatgrass HMWG genes of two lines of partial wheat-wheatgrass hybrids (1670 and 197/2-1-2trs) were cloned and sequenced. The analysis of the sequence data has shown their relationship to HMWG genes of Thinopvrum intermedium (Host) Barkworth & D.R.Dewev and Th. elongatum (Host) D.R.Dewev.

Key words; partial wheat-wheatgrass hybrids, high molecular weight glutenins, sequencing, PCR, protein electrophoresis

Introduction

New genetic resources of wheat should be recruited using wide hybridization due to genetic erosion. Certain species of wheatgrass with different genomic constitution such as *Thinopvrum intermedium (Host) Barkworth & D.RDewey (2n = 42, JSJS), Th. elongatum (Host) D.R.Dewey (2n = 14, J), Th. ponticum (Host) Barkworth & D.RDewev* (2n = 70, JJJJSJS) are donors of agronomically valuable traits and can be relatively easily hybridized with wheat, thus providing the development of wheat-wheatgrass hybrids [16]. As the result of breeding, valuable forms of intermediate wheat-wheatgrass hybrids (WWH) have been developed, their pedigree including different species of wheatgrass, characterized by perennial habit, resistance to pests and diseases, tolerance to salinity and frost. They can be used as independent objects of environmentally sustainable agricultural systems, and as an effective bridge to transfer useful genes from the genome of wheatgrass into wheat [4].

WWH developed in Russia are distinguished by high biodiversity and have been characterized in detail in terms of morphology, developmental biology, farming, and, as far as the most promising lines are concerned, with cytogenetic and molecular genetic approaches [2, 10, 11].

A drawback of WWH as a crop is its low bread-making quality. Significant factors affecting the quality of bread are glutenins. Glutenins consist of high (HMWG) and low molecular weight (LMWG) subunits. The subunits of glutenin are polymerized with intermolecular disulfide bonds, which play a major role in the rheological properties of

wheat dough (elasticity, extensibility, flexibility, dough energy, the time of development, stabilization and early thinning). HMWG of both wheat and wheatgrass can be represented in the genome of WWH.

The influence of HMWG genes of different wheatgrass species on bread-making quality can be either positive or negative.

Most HMWG, which were found in distant relatives of wheat, are smaller as compared with HMWG of wheat [5,6], and therefore, in theory, they could have not only positive but also negative effects on baking quality. Thus, direct introgression without prior impact estimation on quality is inappropriate. Several HMWG orthologous with new structural features have been identified in species of *Aegilops* L,, *Secctle* L,, *Hordeum* L. and *Pseiidoroegnerict* (Nevski) A.Love [1, 7, 8, 13, 14]. However, only a few studies were focused on the allelic composition of HMWG in perennial relatives of wheat so far [12].

The best approach for the evaluation and subsequent introgression of the unique HMW alleles from wild relatives into common wheat is based on the WWH. Using these hybrids, one can evaluate the impact of a unique HMW allele of wheat on bread-making quality, that could not be performed in case of direct detection of that allele in wheatgrass. Moreover, keeping and preserving the detected allele in the WWH is much easier than tracking it in wheatgrass populations. In addition, WWH are a bridge for the introgression of valuable genes directly into wheat genome.

Lines in the studied collection of the WWH differ significantly in bread-making quality between each other. The identification and investigation of HMW genes in wheatgrass and WWH allows understanding the interaction of wheat and wheatgrass genes between each other, as well as their influence on the quality of bread.

The aim of our study was to identify the unique components and analyze the storage proteins in the lines of WWH collection.

Materials and methods

The following 26 lines of WWH from the collection of the Department of Distant hybridization (Main Botanic Garden named in honour of N.V. Tsitsin, Russian Academy of Sciences) were investigated: Ostankinskaya, Zemokormovaya 169, Istra 1, Otrastayushchaya 38, 67 trs, 98 trs, 116 trs, 197/2-1-2 trs, 207/2 trs, 211 trs, 1405, 548, 12, 4082, 4015, 116, 1670, 237, 90, 33, 1689, 77, 1416, 2087, 5542, 4056. As controls, the following wheat cultivars were used: Moskovskaya 39, Mironovskaya 808, Bezostaya 1, Chinese Spring, Novosibirskaya 67, Lutescens 62.

Storage proteins were isolated from individual seeds. Endosperms were crushed and then incubated in SDS-Tris-HCl buffer containing 0.125 M Tris, 2,75% SDS, 10% glycerol, 1% DTT, and 0.005% bromophenol blue for 1 hour at 70 °C, and centrifuged at 14000 ipm for 10 minutes. 20 |il of the supernatants were used for electrophoresis [9].

Proteins were separated on 12.5% polyacrylamide gel using one-dimensional SDS-PAGE electrophoresis. Electrophoresis was performed in a buffer containing
0.25 mM Tris, 19,2 mM glycine, 0.1% sodium dodecyl sulfate in the following mode: 15 mA for 60 minutes, and 30 mA for 4 hours. After electrophoresis the gels were stained with a mixture of Coomassie Brilliant Blue G-250 and R-250 [17].

DNA was isolated from seedlings according to Bematzky & Tanskley [3].

The primers P1 and P2 were used for PCR amplification and subsequent cloning high molecular glutenin genes [15].

Polymerase chain reaction (PCR) was performed using Tetrad 2 Peltier Thermal Cycler (Bio-Rad, USA) under the conditions recommended by the authors of the primers.

25 μI of the reaction mixture contained: IX Taq polymerase buffer (Silex, Moscow), 1.0 U Taq DNA polymerase (Silex, Moscow), 200 μM of each dNTP (Promega), 0.2 μM of each primer, and 100-150 ng of DNA template. PCRproducts were separated by electrophoresis on 2% agarose gel in Tris-borate buffer (TBE). As a DNA weight size marker «100 bp Ladder» («Fermentas», Lithuania) was used. Ligation of the amplified DNA was performed in pGEM®-T Easy Vectors. Sequencing was carried out using the ABI-3130XL sequencer with the M13 primers.

Results and discussion

To characterize the high molecular glutenin genes in wheat-wheatgrass hybrids, P1 and P2 primers were used. The primers were designed on the basis of nucleotide sequences of the conserved regions in the 5 'and 3' ends of the open reading frames of HMWG genes of wheat. [15] We carried out PCR with these primers on the DNA of wheat-wheatgrass hybrids, cultivars of soft wheat, *Th. ponticum*, *Th. elongcttum,* and *Th. intermedium.* The analysis of all above accessions has revealed two types of fragments according to their size: in the range 1800-3000 bp (Figure 1, indicated by green triangular arrows) and less than 1800 bp (Figure 1, indicated by green arrows).

In the wheatgrass accessions, DNA fragments of both types were amplified. In the wheat cultivars, five bands ranging in size from 1800 to 3000 bp were amplified (Figure 1, lane 1, indicated by the red triangle arrows). These five bands were amplified in all analyzed WWH as well.

Amplification of additional wheatgrass bands in WWH lines by PCR with primers P1 and P2

Size of wheat-grass band, bp	1800-3000					Less than 1800 п.н.		
	2700	2500	1800	2700 +2000	2500 +2000	1500	1300	1100
WWH line	Zernokormovaya *, 207/2trs*, 1405*, 4015*, 77, 1416*, 4056	90*	Ostankinskaya *, 67trs*, 12*, 5542*	98trs*	116*	Istra 1, 116trs*, 197/2-1-2trs*, 211trs, 548, 77, 2087, 4056	33	1670*, 1689, 1416

* -lines showing the presence of additional components in the storage protein electrophoresis (SDS-PAGE)

In WWH besides the fragments of wheat type the amplification of additional bands was observed (Figure 1, Table 1).

The size of such fragments in Ostankinskaya, Zemokormovaya 169, 67 trs, 98 trs, 207/2 trs, 1405, 12, 4015, 116, 90, 77, 1416, 5542, 4056 are in the range of 1800 to 3000 bp (Figure 1, indicated by green triangular arrows). Zemokormovaya 169, 207/2 trs, 1405, 4015, 77, 1416, and 4056 had an extra band of about 2700 bp, and lines 77 and 1416 are polymorphic for the presence/absence of extra bands (Figure 1, lanes 24-27). Line 90 had an additional band of about 2500 bp. Ostankinskaya, 67 trs, 12, and 5542 carried an

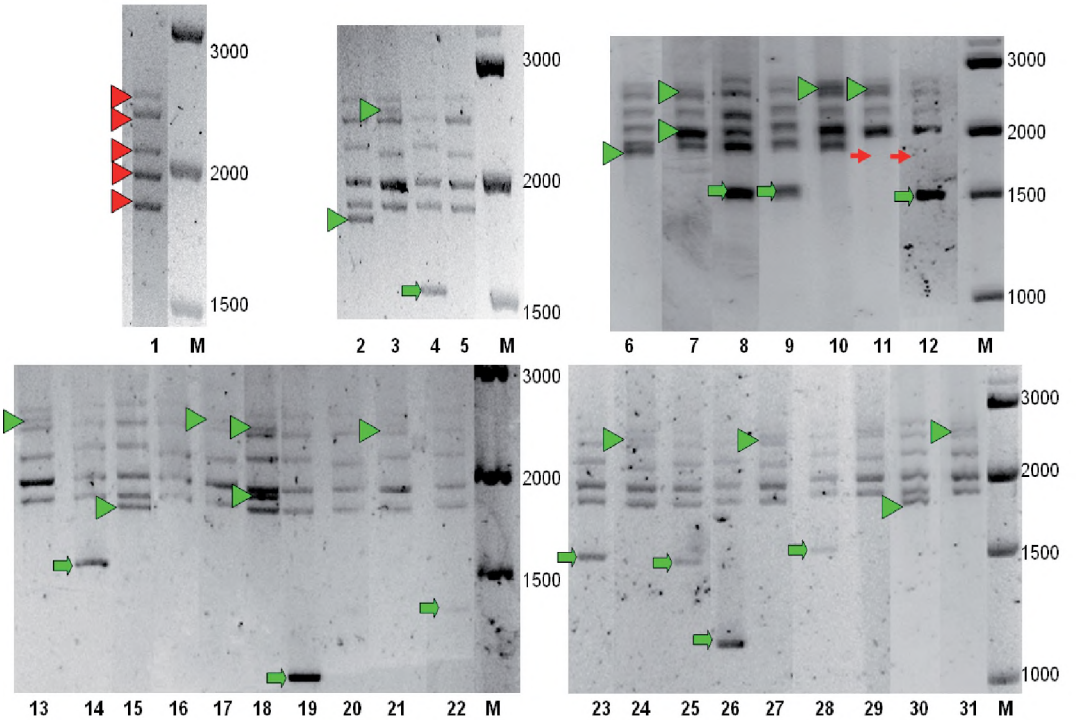

Fig. 1. Electropherograms of the amplification products with primers P1 and P2. Lanes: 1 - soft wheat Lutescens 62, 2 - Ostankinskaya, 3 - Zernokormovaya 169, 4 - Istra 1, 5 - Otrastayushchaya 38, 6-67 trs, 7-98 trs, 8-116 trs, 8-197/2-1 -2 trs, 10, 11 -207/2 trs, 12-211 trs, 13- 1405, 14-548, 15 -12, 16 - 4082, 17 - 4015, 18 - 116, 19-1670 20 -237, 21 - 90, 22 - 33, 23 -1689, 24, 25 - 77, 26, 27, -1416, 28, 29 - 2087, - 5542 30, 31 - 4056, M - DNA ladder. Marking bands arrows: see text.

additional band of about 1800 bp. Lines 98 trs and 116 trs carried two additional bands: the former - around 2700 bp and 2000 bp, the latter - 2500 bp and 2000 bp

Istra 1, 116 trs, 197/2-1-2 trs, 211 trs, 548, 1670, 33, 1689, 77, 1416, 2087 were found to have fragments of less than 1800 bp (Figure 1, indicated by green arrows). Bands of approximately 1500 bp were amplified in Istra 1, 116 trs, 197/2-1-2 trs, 211 trs, 548, 77, 2087, 4056. At the same time, lines 77 and 2087 were polymorphic for the presence/absence of the band. An additional band of approximately 1300 bp was detected in line 33. Lines 1670, 1689, and 1416 had additional bands of about 1100 bp. Thus, lines 77 and 1416 possessed two types of bands: more and less than 1800 bp.

As these additional fragments amplified by PCR are not specific to wheat, they might be amplified from DNA sequences of wheatgrass.

The bands of wheat type of 2000 bp were absent in lines 211 trs and 207/2 trs (Figure 1, lanes 11, 12, indicated by a red arrow). This may be due either to a deletion in this locus, or to lack of wheat chromosomes.

Thus, we have analyzed 26 WWH lines, 23 of them showing the amplification of DNA fragments typical to high molecular weight glutenin of wheatgrass. The polymorphism of amplified fragment size between the studied lines was observed.

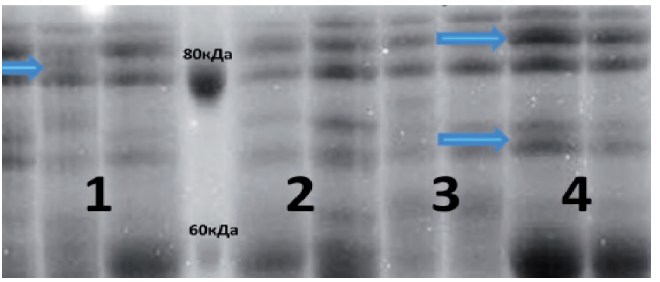

Fig. 2. One-dimensional electrophoresis of storage proteins in partial wheat-wheatgrass hybrids. Unique storage proteins for wheat genome are marked by arrows. 1 - Ostankinskaya 2 - Istra 1 , 3 - Otrastayushchaya 38 4 - Zernokormovaya 169

A hexaploid wheat genotype could contain six different high molecular weight glutenin subunits, but silencing some of these genes leads to the formation of different combinations of HMW subunits - from three to five subunits in hexaploid wheat and one to three in durum wheat. Consequently, HMWG genes with wheatgrass origin identified by us could be silent genes. Therefore, the next stage of our work was to analyze the collection of WWH of different levels of breeding using storage protein electrophoresis (SDS-PAGE). Examples of SDS-analysis of high molecular weight glutenin subunits of WWH are presented in Figure 2. The identification of unique storage proteins was performed by comparing the results obtained with the subunits composition of control cultivars (Chinese Spring, Mironovskaya 808, Novosibirskaya 67, Moscovskaya 39). Determining which particular component of storage proteins belongs to the wheatgrass appeared to be impossible because in almost all pedigrees of WWH several different types of wheatgrass had been used. To compare the unique components directly with the storage protein of wheatgrass is also difficult, because wheatgrass proteins are extremely polymorphic. Therefore, the identified components which were different from components of wheat were noted as additional.

As a result of SDS-PAGE analysis, we have revealed that Ostankinskaya, Zemokormovaya 169, 67 trs, 98 trs, 207/2 trs, 1405, 12, 4015, 116, 90, 1416, 5542, which amplified additional bands in the range of 1800 to 3000 bp, at the same time carried the components of HMW subunits differing in size from HMW subunits of wheat. Among the lines with additional bands smaller than 1800 bp amplified from the primers for HMW genes only 116 trs, 197/2-1-2 trs and 1670 have components of storage proteins different from those of wheat.

Additional HMW components have not been revealed in 4056, Istra 1, 211 trs, 548, 33, 1689, 77, 1416, 2087. It could be due to gene silencing or that HMW subunits of wheatgrass origin have molecular weight close or identical to HMW subunits of wheat, and they cannot be separated from each other using one-dimensional electrophoresis of storage proteins.

To study the identified genes and confirm our results (that the additional bands are amplified from HMWG genes of wheatgrass), we have cloned, transformed and sequenced additional bands amplified with the primer pair P1/P2 on WWH line 1670 (the size of about 1100 bp) and 197/2-1-2 trs (around 1500 bp).

As a result, two different nucleotide sequences have been obtained: of 1152 bp in line 1670 and 1352 bp in line 197/2-1-2 trs. (Figure 3).

BLAST-analysis has displayed a high degree of homology of the analyzed fragments to the published genes of high molecular glutenin genes of wheatgrass. Thus, these data entirely confirm that the bands additionally amplified with the P1 and P2 primers on the WWH lines are HMWG genes of wheatgrass origin. Interestingly, the gene identified in

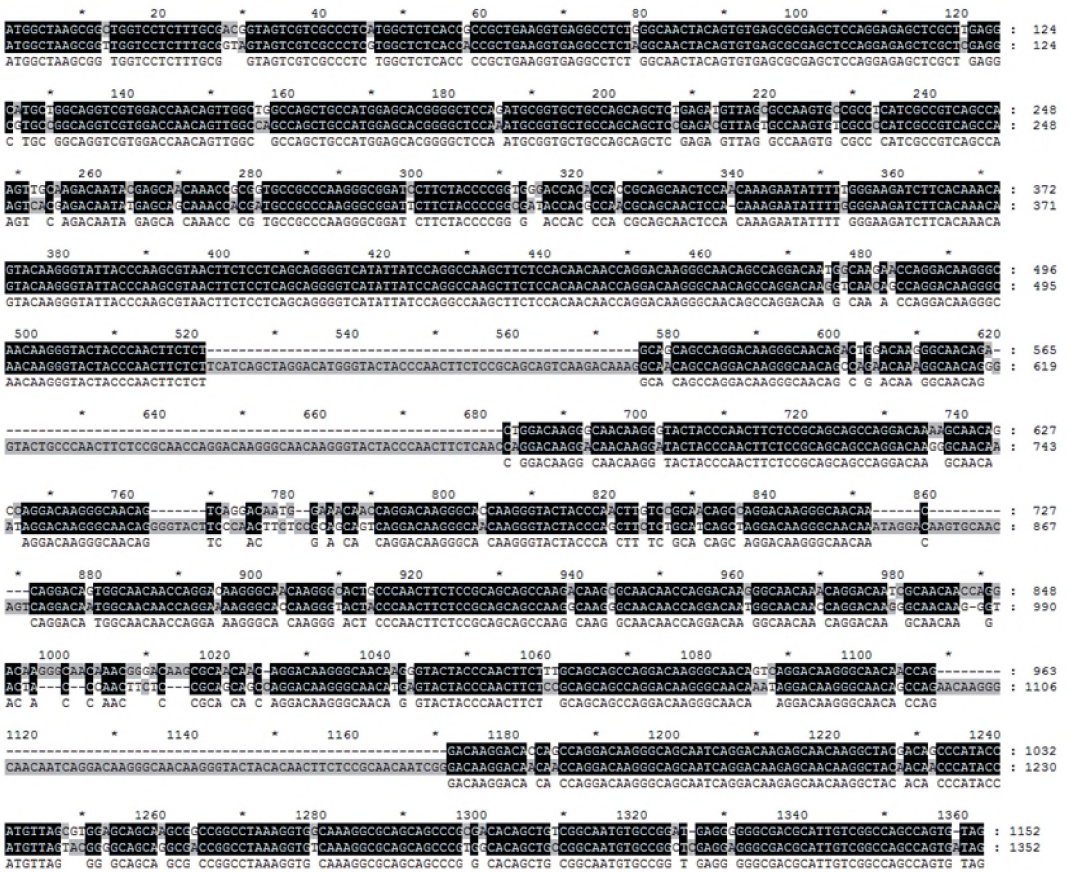

Fig. 3. Alignment of the nucleotide sequences obtained from lines 1670 (top) and 197/2-1-2 trs (bottom)

1670 showed the greatest homology and clustered to the HMWG gene of *Th. intermedium,* while the gene detected in 197/2-1-2 trs, clustered to the gene of *Lophopyrum elongatum* (Host) A.Love (synonym *Th. elongatum)* (Figure 4).

According to the results of the long-term estimation, Ostankinskaya, 12, 4015, 5542 were close to the standard wheat cultivar (Moscovskaya 39) in a number of parameters

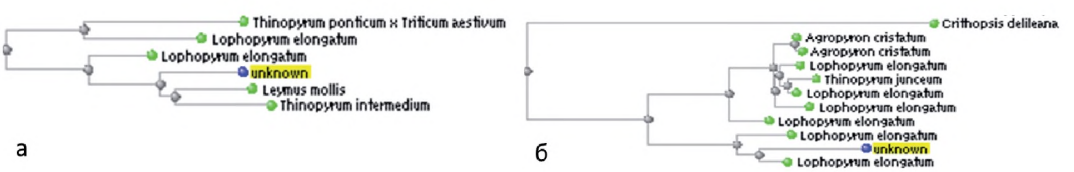

Fig. 4. Clustering HMWG genes of wheatgrass (using fast minimum evolution) identified in 1670 (a) and 197/2-1-2 trs (b). The studied genes are highlighted in yellow

(sedimentation coefficient, total baking score, etc.) in some years of the experiment. These lines are considered to be a promising material for breeding on quality (VI. Belov, unpublished data). Moreover, we have shown the presence of additional amplification of DNA fragments specific to the high molecular weight glutenin genes of wheatgrass origin and additional storage protein components by one-dimensional protein electrophoresis. Consequently, these lines are interesting as objects to study the effects of wheatgrass HMWG on the storage protein composition and quality of bread.

In conclusion, our research has revealed the unique components of the storage proteins in the following lines: Ostankinskaya, Zemokormovaya 169, 67 trs, 98 trs, 207/2 trs, 1405, 12, 4015, 116, 90, 1416, 5542, 116 trs, 197/2- 1-2 trs, 1670. The given components were close to high molecular weight glutenin of wheat in their absolute molecular weight, which increases the possibility of their favorable effect on the baking quality of wheat. Twenty six lines of WWH have been analyzed using primers amplifying entire high weight molecular glutenin genes. The DNA fragments typical to HMWG genes of wheatgrass were amplified in twenty three lines.

The polymorphism of amplified fragment size between the studied lines was observed. The wheatgrass genes of high molecular weight glutenin of two WWH lines were cloned and sequenced.

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References

1. Analysis of HMW glutenin subunits and their coding sequences in two diploid *AegHops* species / Z. Liu [et al] // Theor. Appl. Genet. 2003. Vol. 106. № 8. P. 1368-1378.

2. *Belov V.I., Ivanova L.P.* Improvement of productivity of octoploid partial wheat-wheatgrass hybrids Uluchsheniye productivnosti oktoploidnykh promezhutoclmykh PPG // Otdalennaya gibridizatsiya. Rezultaty issledovainya / ed. VI. Semenov. Moscow, 2001. P. 166-177.

3. *Bernatzkv R., Tanksley S.D.* Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences // Genetics. 1986. Vol. 112. № 4. P. 887 -898.

4. Characterization of a partial *wheat-Thinopyrum intermedium* amphiploid and its reaction to fungal diseases of wheat / Z.-J. Chang [et al.] // Hereditas. 2010. Vol. 147. № 6. P. 304-312.

5. Characterization of high-molecular-weight glutenin subunit genes from *Elytrigia elongata* / J.R. Wang [et al.] // Plant Breeding. 2006. Vol. 125. № 1. P. 89-95.

6. Characterization of HMW prolamines and their coding sequences from *Crithopsis delileana* / Z.-F. Guo et al. // Hereditas. 2005. Vol. 142. P. 56-64.

7. Characterization of the HMW glutenin subunits from *Aegilops searsii* and identification of a novel variant HMW glutenin subunit / X. Sun [et al.] // Theor. Appl. Genet. 2006. Vol. 113. № 4. P. 631-64L

8. *De Bustos A., Jouve N.* Characterisation and analysis of new HMW-glutenin alleles encoded by the Glu-Rl locus of *Secale cereale II* Theor. Appl. Genet. 2003. Vol. 107. № 1. P. 74-83.

9. *Dekova T.* The Gluten-a big natural biopolymer genetic determination and function // Biotechnology and Biotechnological Equipment. Supplement General and Applied Genetics. 2005. Vol. 19. P. 11-18.

10. *Kroupin P.Yu., DivashukM.G., Belov VI., Glukhova L.I., Alexandrov O.S., Karlov G.I.* Comparative Molecular Cytogenetic Characterization of Partial Wheat-Wheatgrass Hybrids / P.Yu. Kroupin et al. // Russian Journal of Genetics. 2011. Vol. 47. № 4. P. 432-437.

11. *Kroupin PYu., Divashuk M.G., Fesenko I.A., Karlov G.I.* Adaptation of microsatellite SSR-markers in wheat to genome analysis of intermedium wheatgrass, wheat grass and wheat-wheatgrass hybrids / P.Yu. Kroupin [et al.] // Izvestiya TSKhA,. 2011. № 3. P. 49-57.

12. *Liu S., GaoX.,Xia G.* Characterizing HMW-GS alleles of dec a p 1 o **i** d .1 *grop mm elongatum* in relation to evolution and wheat breeding // Theor. Appl. Genet. 2008. Vol. 116. № 3. P. 325-334.

13. *Piston F., Shew my PR., Bammo F. D* Hordeins of *Hordeum chilense:* a novel source of variation for improvement of wheat//Theor. Appl. Genet. 2007. Vol. 115. № 1. P. 77-86.

14. Structural organization of the barley D-hordein locus in comparison with its orthologous regions of wheat genomes / Y.Q. Gu [et al.] // Genome. 2003. Vol. 46. № 6. P. 1084-1097.

15. Structural variation and evolutionary relationship of novel HMW glutenin subunits from *Elymus glaucus* / Q.-T. Jiang [et al.] // Hereditas. 2010. Vol. 147. № 3. P. 136-141.

16. *Tsitsin N.V.* Mnogoletnyaya pshenitsa (Perennial wheat). Moscow: Nauka (Science), 1978. 288 p.

17. Wheat Applied Genomics - Gluten strength [Online]. URL: http://maswheat.ucdavis.edu/protocols/gluten/index.htm.

МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЙАНАЛИЗ СОСТАВА ЗАПАСНЫХ БЕЛКОВ В ОБРАЗЦАХ КОЛЛЕКЦИИ ПШЕНИЧНО-ПЫРЕЙНЫХ ГИБРИДОВ

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Аннотация: цель исследования состояла в изучении генов запасных белков пырейного происхождения у пшенично-пырейных гибридов (ППГ). С помощью ПЦР выявлено 23 образца ППГ, несущих гены высокомолекулярных глютенинов (ВМГ) пырейного происхождения. Методом белкового электрофореза показано, что минимуму 15 из 23 образцов эти гены экспрессируются. У двух образцов ППГ (1670 и 197/2-l-2trs) гены высокомолекулярных глютенинов пырейного происхождения были клонированы и секвенированы. Анализ полученных сиквенсов показал родство к генам ВМГ Thinopvrum intermedium (Host) Barkworth & D.R.Dewey и Th. elongatum (Host) D.R.Dewey

Ключевые слова: промежучтоные пшенично-пырейные гибриды, высокомолекулярные глютенины, секвенирование, ПЦР, электрофорез белков.

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