https://doi.org/10.15407/scine19.06.031

MORGUN, B. V.[1, 2] (https://orcid.org/ 0000-0001-7041-6894/),
SANDETSKA, N. V.[1] (https://orcid.org/ 0000-0002-0558-2295/),
and VELYKOZHON, L. H.[1, 2] (https://orcid.org/ 0000-0002-5935-9363/)

[1] Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine,
31/17, Vasylkivska St., Kyiv, 03022, Ukraine,
+380 44 257-5160, plant@ifrg.kiev.ua
[2] Institute of Cell Biology and Genetic Engineering of the National Academy
of Sciences of Ukraine,
148, Academika Zabolotnoho St., Kyiv, 03143, Ukraine,
+380 44 526 7104, molgen@icbge.org.ua

THE EFFECT OF THE *Gpc-B1* GENE ON THE PROTEIN CONTENT OF SOFT WINTER WHEAT GRAIN AGAINST THE BACKGROUND OF GENETIC ENVIRONMENT OF UKRAINIAN VARIETIES

Introduction. *The issue of improving grain quality is an urgent problem of wheat breeding. The transfer of genes from wild relatives is one of the directions of genetic improvement of wheat. The Gpc-B1 gene is of particular interest in this direction.*

Problem Statement. *Using modern DNA marker systems to determine the Gpc-B1 gene from T. turgidum ssp. dicoccoides makes it possible to create new promising innovation varieties with increased protein content in combination with high economic and valuable properties in a short period of time.*

Purpose. *The purpose of our research is to determine the effect of the Gpc-B1 gene in the genetic environment of the Ukrainian variety Kuyalnyk on the grain protein content, yield, and bread-making characteristics.*

Material and Methods. *The Gpc-B1 gene has been detected by the method of multiplex polymerase chain reaction (PCR). The source of the Gpc-B1 gene, the Glu-Pro line, has been crossed with the Kuyalnyk variety (Gpc-B1 × Kuyalnyk). The protein content in grain has been measured by infrared spectrometry (NIR); the sedimentation index has been determined by the SDS-30 method, on an automatic device.*

Results. *Dominant and codominant molecular genetic systems of DNA markers have been developed to detect the Gpc-B1 gene from Triticum turgidum ssp. dicoccoides in soft winter wheat lines. The analysis of the grain of plants of generations F_5—F_9 has shown that the Gpc-B1 gene causes a 3% increase in protein content as compared with the original Kuyalnyk variety. The productivity of lines carrying the Gpc-B1 gene has been analyzed in field conditions.*

Citation: Morgun, B. V., Sandetska, N. V., and Velykozhon, L. H. (2023). The Effect of the Gpc-B1 Gene on the Protein Content of Soft Winter Wheat Grain against the Background of Genetic Environment of Ukrainian Varieties. *Sci. innov.*, 19(6), 31—39. https://doi.org/10.15407/scine19.06.031

Conclusions. *The results of our research have shown that the influence of the Gpc-B1 gene on the grain yield is practically absent against the background of the genetic environment of the Kuyalnyk zoned Ukrainian variety. The influence of this gene on the baking properties of soft wheat has been studied. The lines with the Gpc-B1 gene and without it have almost the same sedimentation rate. We have created a modern breeding material of soft winter wheat with increased protein content in the grain in combination with high economic and valuable properties, which is ready for the state variety tests.*

Keywords: Triticum aestivum L., Gpc-B1 gene, Triticum turgidum ssp. dicoccoides, molecular markers, grain protein content, and sedimentation rate.

Increasing the protein content of wheat grain remains one of the strategic tasks of modern breeding. However, it is a complex polygenically determined trait, largely dependent on agroclimatic growing conditions and, as a result, hard to control and manage in the breeding process [1]. According to the UN forecast, from 2000, the Earth's population will grow by 50% in the next half century and will reach about 9.5 billion people by 2050. In order to feed such a mass of the population, the agricultural products shall increase by 60% [2]. Among the nutritional factors, vegetable protein has now and will have strategic biological importance in the future. Today, the share of total vegetable protein consumed by the Earth's population is 57%. At least half of the total amount of vegetable protein during the last decades has been stably supplied by wheat [3]. Therefore, the issue of increasing both the amount of wheat protein (content in the grain) and its quality (technological and biological value) is a priority in the genetic research of wheat culture in the near future. Along with protein, trace elements are extremely important in human nutrition, and more than half of the world population suffers from their deficiency in daily food [4].

An important characteristic that determines the quality of wheat flour is the structural grain composition. It contains 13—17% of the membranes, 2—3% of the embryo, and 81—84% of the endosperm. The special properties of wheat are related to the protein complex formed by gliadins, glutenins, albumins, and globulins. In wheat grain, the storage proteins — gliadin and glutenin — have a share of 80—85% in the total protein content. Albumins and globulins act as structural and enzymatic proteins of the aleurone layer and embryo. The storage proteins carry the main functional load in terms of their influence on the quality of gluten. Glutenins are polymerizable through the formation of intermolecular disulfide bonds that create the macromolecular framework of gluten and are responsible for the elasticity and firmness of the dough. Gliadins occupy about 40—50% of the total protein and contribute to the elasticity and strength of gluten [1].

The protein content is an important characteristic of the quality of wheat grain, which is regulated by DSTU 3768:2019. According to the government standard, wheat of the 1st class shall contain at least 14% of the total protein in the grain, that of the 2nd class shall have at least 12.5%, and that of the 3rd class shall possess at least 11% of the total protein. However, in recent decades, instead of an increase in yield, the grain quality, including the protein content index has been deteriorating [5].

The *Gpc-B1* gene is of particular interest in this direction. In wild wheat, dicotyledons *T. turgidum* ssp. *dicoccoides* from the national germplasm funds of Israel, in chromosome 6B, there has been identified wild-type gene *Gpc-B1* (grain protein content, NAM-B1) ($2n = 4x = 28$) with genomic formula $A^u A^u BB$, which significantly increases the content of protein and several key trace elements in the grain without affecting the yield, due to the acceleration of the physiological aging of plants and the coding of a transcription factor that stimulates the remobilization of nitrogen, iron, and zinc from the vegetative organs into the grains [1, 6—9]. In most modern varieties of wheat, there is no *Gpc-B1* gene or it does not function (mutated) [10].

Lines carrying the *Gpc-B1* gene have been successfully used in the breeding program aimed at

increasing the content of total protein and key trace elements in wheat grain [11]. The *Gpc-B1* gene has been cloned and studied in detail both in terms of molecular structure and functionality [12].

The detailed study of the *Gpc-B1* gene as a factor encoding a NAC-domain protein has made it possible to clearly define its position in chromosome 6B, identified as a 7400-bp sequence located between marker loci *Xucw* 109 and *Xuhw* 106. Marker locus *Xuhw* 84, orthologous of the rice gene *OSJNBa002E05.19-1*, appeared to be closely linked to *Gpc-B1*. It can be used as a sufficiently reliable DNA marker for the detection of *Gpc-B1* in breeding populations [13, 14].

It is important to emphasize that unlike other previously identified genes that affect the grain protein content, *Gpc-B1* is a unique gene. Firstly, because of its strong positive influence on both the content of total protein and, at the same time, on the content of key trace elements in wheat grain. Secondly, for the mechanism of its expression, which manifests itself morphologically as phenomenon of physiological aging of plants [1, 9].

In the course of the series of experiments in different world countries, on different genetic backgrounds and under contrasting growing conditions, the high efficiency of using the *Gpc-B1* gene in breeding programs with the aim of increasing the protein content of grain and improving its technological and consumer value has been proven [1, 12, 15]. A strong positive effect of the *Gpc-B1* gene and the extraexpression of high molecular weight glutenin subunits *Glu-A1x2**, *Glu-D1x*5 on the grain hardness, grain protein content, and grain quality has been reported [16]. The research results have proven that the *Gpc-B1* gene transferred from wild spelled *Triticum turgidum* ssp. *dicoccoides* into the new genetic environment of highly productive Ukrainian variety *Kuyalnyk* positively influences the accumulation of the total protein in the grains of soft hexaploid winter wheat. All experimental lines carrying the *Gpc-B1* gene have a protein content that is higher by 14%, on average, as compared with the parent variety [17].

The *Gpc-B1* gene is also responsible for accelerated senescence of leaves and high content of important trace elements such as Zn, Mn, and Fe in wheat grain [1]. The effect of the locus has been studied and shown a significant increase as compared with the baseline in the grain content of zinc (60 mg/kg vs. 47.5 mg/kg), iron (44.2 mg/kg vs. 35.9 mg/kg), manganese (53.9 mg/kg vs, 40.9 mg/kg), and protein (14.4% vs. 10.8%) [12, 14, 18].

The purpose of this research is to determine the effectiveness of the *Gpc-B1* gene in the genetic environment of Ukrainian-bred variety *Kuyalnyk* on the grain protein content, yield, and bread-making properties.

Field experiments were conducted at the experimental field of the IPPG of the NAS of Ukraine in 2012—2022. The source of the *Gpc-B1* gene, the Glu-Pro line [14], is crossed with the *Kuyalnyk* variety (*Gpc-B1* × *Kuyalnyk*). The population of F_2–F_4 hybrids and the resulting homozygous lines have been tested on 10—20 m^2 plots, in 3—4-fold repetitions. Lines (F_5—F_9) are generated by the pedigree method during ear-by-ear (earrow) sowing. Homozygous lines with the *Gpc-B1* locus have been observed by phenological indicators and counted according to the generally accepted methods [19].

The protein content in grain has been measured by infrared spectrometry (NIR). Grain weighing 40 g is ground by a *Perten* LM 3100 laboratory mill (Sweden). The protein content of flour has been determined by a *Perten Inframatic* 8600 device (Sweden), according to the manufacturer's recommendations.

The strength of flour, i.e. the sedimentation index determined by the SDS-30 method (SDS is sodium dodecyl sulfate) has been evaluated indirectly by an automatic device with software control [20]. For this, an average flour sample weighing 3.2 g is taken and poured with 10 ml of 4% acetic acid. After that, the flour sample is placed in a water bath heated to 30 °C and stood for 30 min; 20 ml of 4% glacial acetic acid solution and 2% SDS solution are added to each sample up to the mark of 100 cm^3. The flour samples are placed in

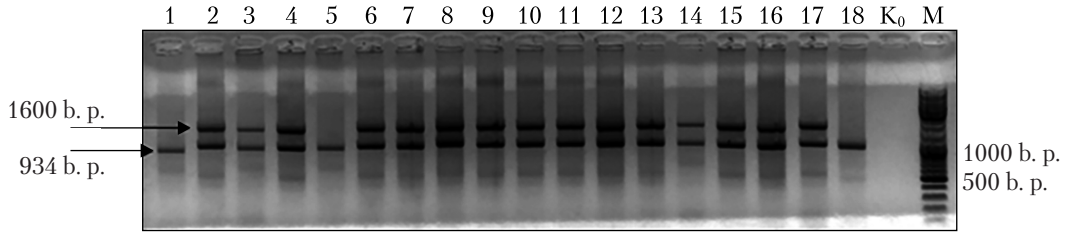

Fig. 1. Electrophorogram of the results of multiplex PCR for detecting the presence of the *Gpc-B1* gene and reference gene *TaTM20*. Lanes 1—16 — the wheat samples of generation F_2—F_4; 17 — the *Gpc-B1* gene donor; 18 — the *Kuyalnyk* wheat variety; K_0 is the negative reference without DNA; M is the molecular weight marker of GeneRuler™ DNA Ladder Mix

the automatic device, the program of rotation cycles runs, and in 16 min the readings are taken.

The *Gpc-B1* gene is detected by the method of multiplex polymerase chain reaction (PCR). The primers used in the study are listed in Table 1.

The total DNA is isolated by the CTAB method. The multiplex PCR to detect the *Gpc-B1* gene with *TaTM20* as a reference gene is as follows: 4 min denaturation at 94 °C; 35 cycles: 30 s denaturation at 94 °C, 1 min renaturation at 64 °C, 1 min 40 s elongation at 72 °C, and 5 min final elongation at 72 °C. The final concentration of primers in the reaction is 0.5 μM. The PCR to detect the *Gpc-B1* gene (codominant system) is as follows: 4 min denaturation at 94 °C; 10 cycles: 30 s denaturation at 94 °C, 30 s renaturation at 65 °C (the temperature decreases by 1 °C in each cycle), 20 s elongation at 72 °C; then 25 cycles: 30 s denaturation at 94 °C, renaturation 20 s at 55 °C, 20 s elongation at 72 °C; 5 min final elongation at 72 °C. The final concentration of primers in the reaction is 0.5 μM.

The amplification products are separated by electrophoresis in a 1.5% agarose gel, with the use of ethidium bromide as a dye.

The experimental data are statistically processed with the use of the *Microsoft Excel*.

TESTING THE WHEAT SAMPLES FOR THE *GPC-B1* GENE

The first stage of our research is to test the wheat samples of the F_2—F_4 generation for the presence of the *Gpc-B1* gene using a dominant system of molecular genetic markers. Amplicons of 1600 bp are expected for the *Gpc-B1* gene and 934 bp. for reference gene *TaTM20*. A typical electrophoreogram is presented in Fig. 1.

The results of the analysis of F_2—F_4 hybrids with the use of the dominant marker system have allowed the selection of lines with the *Gpc-B1* gene. The mass selection of elite plants (300—500 pcs.) that meet the highest requirements for a complex of economic and valuable properties among the populations of F_2—F_4 hybrids has been done.

Table 1. **The Primers Used in the Research**

No.	Target gene, allele	Sequence	Amplicon size, bp.
1	*Gpc-B1*	5′-TTCACAAACTAAGGGGAGGGA-3′ 5′-CTACCATCGAAAGTTGATAGGGA-3′ [21]	1600
2	*Gpc-B1*	5′-TCTCCAAGAGGGGAGAGACA-3′ 5′-TTCCTCTACCCATGAATCTAGCA-3′ [22]	122, 126
3	*TaTM20*	5′-AAGGGTTGCTCCTCTTCGCGATCTTG-3′ 5′-GTACATGCCAGCACCGTATGGATTG-3′ [23]	934

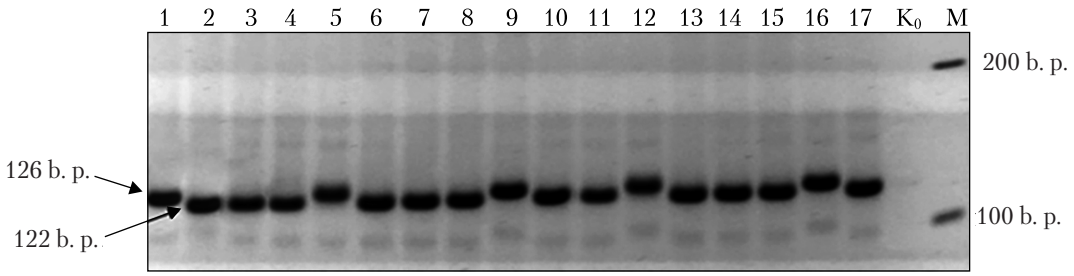

Fig. 2. Electrophorogram of DNA amplification products of wheat samples with primers to the *Gpc-B1* gene. Lanes 1—15 — the lines; 16 — the *Kuyalnyk* variety; 17 — the *Gpc-B1* gene donor Glu-pro line; K_0 is the negative reference, without DNA; M is the molecular weight marker of GeneRuler™ DNA Ladder Mix

A codominant molecular genetic marker system has been used to test the lines of the F_5 hybrid generation for the presence of the *Gpc-B1* gene. The presence of amlicone 122 bp. indicates allele of the *Gpc-B1* gene from *Triticum turgidum* ssp. *dicoccoides*, while amplicon of 126 bp. means the presence of inactive allele from *T. aestivum*. A typical electrophorogram is presented in Fig 2. The codominant system of molecular genetic markers tested 35 lines of F_5 hybrids selected given the phenotypic characteristics. The presence of functional allele of the *Gpc-B1* gene is monitored annually until the F_9 generation. It has been found that 28 lines have functional allele of the *Gpc-B1* gene from *T. turgidum* ssp. *dicoccoides*, whereas 7 lines do not have this allele.

EFFECT OF THE *Gpc-B1* GENE ON THE GRAIN PROTEIN

It is especially important to study the effects of the *Gpc-B1* gene on the grain quality and yield under different climatic conditions of wheat cultivation. We have conducted our research in the forest-steppe zone of Ukraine. The content of total protein in wheat lines of F_5—F_9 generation has been determined by the method of infrared spectrometry (NIR). For the analysis, 35 lines that were previously tested for the presence of the *Gpc-B1* gene have been selected. Among the analyzed 35 lines: 2 have a protein content above 18%; 19 lines have that of above 16.0%; and 14 lines have that of above 14%. The highest protein content has been found in line No. 16 (18.1%), while the lowest one has been reported for lines No. 1 and No. 6 (14.6%). On average, in lines with the presence of functional allele of the *Gpc-B1* gene, there has been observed an increase of 3.0% relative to mother variety *Kuyalnyk*. At the same time, the protein content of mother variety *Kuyalnyk* is 13.6%, while the parental line of the *Gpc-B1* gene donor is 16.9% (Fig. 3). Particular attention should be paid to lines No. 10, No. 15, No. 16, No. 17, No. 18, and No. 19, in which the protein content exceeds 17%. In the line that does not have the *Gpc-B1* gene, the average protein content makes up 15.2% that is 1.4% less as compared with the lines that have the *Gpc-B1* gene. The results obtained by us have been confirmed by literature data. Tabbita et al. [12], while summarizing the results of 25 studies, have found that among the analyzed samples, 91% of the studied

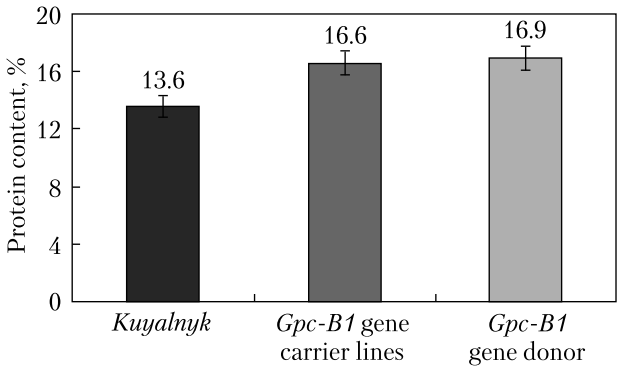

Fig. 3. The protein content in grain by variety (average for 2019—2022)

Table 2. **The Results of the Analysis of the Qualities of Wheat Lines (*Gpc-B1* × *Kuyalnyk*) (average for 2019–2022)**

No of lines	Protein content, %	Presence of the *Gpc-B1* gene	Yield, t/ha	SDS-30, ml
1	14.6	–	8.1	94.0
2	15.4	–	7.7	95.0
3	15.6	–	7.7	93.0
4	15.6	–	7.6	92.5
5	15.2	–	8.3	87.0
6	14.6	–	8.6	95.0
7	15.5	–	9.1	94.5
8	15.9	+	6.8	94.5
9	16.9	+	7.9	93.5
10	17.4	+	7.9	94.5
11	16.3	+	8.3	92.0
12	16.7	+	8.1	94.0
13	16.7	+	7.9	94.0
14	16.0	+	7.8	57.5
15	18.0	+	7.3	93.0
16	18.1	+	7.1	96.0
17	17.4	+	8.2	93.5
18	17.5	+	7.7	93.0
19	17.3	+	8.1	92.5
20	15.8	+	7.3	95.5
21	15.8	+	7.5	95.0
22	16.8	+	7.8	90.0
23	16.2	+	8.1	84.0
24	16.6	+	9.0	85.0
25	15.9	+	8.3	88.0
26	16.0	+	8.3	95.0
27	16.0	+	8.2	95.5
28	16.8	+	8.3	94.5
29	16.5	+	9.3	94.5
30	16.9	+	8.3	95.0
31	16.6	+	8.3	94.0
32	15.9	+	8.9	95.0
33	15.2	+	8.0	91.5
34	15.7	+	8.6	94.0
35	16.5	+	8.2	92.0
Kuyalnyk variety, reference	13.6	–	8.2	91.5
Donor line of the *Gpc-B1* gene	16.9	+	3.1	92.0
$HIP_{0.5}$	0.7	–	0.3	5.2

Note: "+" is the presence of the *Gpc-B1* gene from *Triticum turgidum* ssp. *dicoccoides;* "–" is the presence of the *Gpc-B1* from *T. aestivum*.

wheat lines with functional wild-type *Gpc-B1* allele have an average protein content by 21.8% higher than the lines with non-functional allele.

In the other 9% of the studied lines, the difference in the protein content between the compared variants of the experiment is insignificant. The obtained results have indicated that the *Gpc-B1* gene transferred from wild spelled to highly productive Ukrainian variety *Kuyalnyk* positively affects the protein content of the grain. The studied lines are valuable genetic material for the further breeding and the creation of new high-quality wheat varieties with a high protein content.

EFFECT OF THE *Gpc-B1* GENE ON THE YIELD

Yield is one of the main factors for evaluating wheat varieties. The functional allele of wild-type *Gpc-B1* is an atypical genetic factor for soft wheat, which positively affects the protein content of the grain (the parental line of the donor of the *Gpc-B1* gene is short and low-yielding, but has a high protein content, the second parental form — the *Kuyalnyk* variety in terms of grain quality — is extra-strong, high-yielding, entered in the State Register of Plant Varieties of Ukraine in 2003, suitable for cultivation in steppe and forest-steppe zones). At the same time, there is an inverse trend between the protein content and the wheat yield, which is the basis for sudying the relationship between the *Gpc-B1* functional allele and the yield. We have compared the yield of the created lines with the parental forms: the *Kuyalnyk* variety (yield 8.2 t/ha) and the *Gpc-B1* gene donor line (yield 3.1 t/ha). Among the 28 wheat lines carrying the functional allele of the *Gpc-B1* gene, there are 12 lines with the yield higher than that of the *Kuyalnyk* variety: line No. 11 (8.3 t/ha), No. 24 (9.0 t/ha), No. 25 (8.3 t/ha), No. 26 (8.3 t/ha), No. 27 (8.2 t/ha), No. 28 (8.3 t/ha), No. 29 (9.3 t/ha), No. 30 (8.3 t/ha), No. 31 (8.3 t/ha), No. 32 (8.9 t/ha), No. 34 (8.6 t/ha), and No. 35 (8.2 t/ha). The average yield of the 28 lines with the *Gpc-B1* gene is 8.1 t/ha, while the average yield of the 7 lines that do not contain this gene is 8.2 t/ha (Table 2).

Thus, the results of our research have indicated that the influence of the *Gpc-B1* gene against the background of the genetic environment of zoned Ukrainian variety *Kuyalnyk* on the grain yield is practically negligible. The results obtained by us have been confirmed by the literature data. Tabbita et al. [12] have summarized the data of 15 studies on the influence of the *Gpc-B1* gene on the yield. Among these studies, in 79% of the cases, there is no negative effect of the *Gpc-B1* on the yield, in 17% there is a positive correlation between the *Gpc-B1* gene and the yield. The maximum increase in the yield as a result of the *Gpc-B1* gene introgression is 0.634 t/ha, while in 4% of the studied cases, there is a decrease in the yield by 0.363 t/ha.

EFFECT OF THE *Gpc-B1* GENE ON THE SEDIMENTATION (SDS-30)

An increase in the protein content, as a result of the expression of the wild-type allele of the *Gpc-B1* gene, can affect the baking properties of common wheat. To solve this issue, we have analyzed the baking quality of wheat by the SDS-30 method. The twenty-five studied lines of wheat carrying the *Gpc-B1* gene have a sedimentation rate as high as that of original *Kuyalnyk* variety, from 88 to 95 ml. High SDS-30 sedimentation index indicates high baking quality of the flour. It should be noted that the genotypes with the *Gpc-B1* + gene as compared with the genotypes without it have almost the same sedimentation index of 92 ml.

The lines created by us (*Gpc-B1* × *Kuyalnyk*) have no obvious negative phenotypic deviations from the characteristics of the *Kuyalnyk* variety. A feature of such lines is somewhat accelerated (by 2—3 days) ripening and dying of leaves on plants.

CONCLUSIONS

1. The introgression of the *Gpc-B1* gene functional allele into the genetic environment of Ukrainian varieties has led to an increase in the protein content by 3% as compared with original variety *Kuyalnyk*.

2. The dominant and codominant molecular genetic systems of DNA markers have been developed to detect the *Gpc-B1* gene in soft winter wheat plants.

3. The applied methods of marker-assisted selection have ensured the creation of promising wheat breeding material that is ready to be transferred to the state variety testing, with a high protein content in the grain in combination with high economic and valuable plant properties.

FUNDING OF THE RESEARCH

The research is funded by state project No. 6541030 of the National Academy of Sciences of Ukraine.

ACKNOWLEDGEMENTS

We express our gratitude to Full Member of the NAS of Ukraine V. V. Morgun, Associate Member of the NAS of Ukraine O. I. Rybalka, for guidance and advice and to Distinguished Prof. J. Dubcovsky for the source material.

REFERENCES

1. Rybalka, O. I., Morhun, B. V., Polishchuk, S. S. (2018). *Gpc-B1* (*NAM-B1*) gene as a new genetic resource in wheat breeding for increasing grain protein and micronutrient content. *Fiziolohiya roslyn i henetyka*, 50(4), 279—298 [in Ukrainian]. https://doi.org/10.15407/frg2018.04.279
2. Alexandratos, N., Bruinsma, J. (2012). *World agriculture: towards 2030/2050. The 2012 revision*. Rome.
3. Henchion, M., Hayes, M., Mullen, A., Fenelon, M., Tiwari, B. (2017). Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. A review. *Foods*, 6(7), 53. https://doi.org/10.3390/foods6070053.
4. Zhao, F., McGrath, S. (2009). Biofortification and phytoremediation. *Curr. Opin. Plant Biol*., 12, 373—380.
5. Pokhylko, S. Yu., Schwartau, V. V., Pochynok, V. M., Mykhalska, L. M., Duhan, O. M., Morgun, B. V. (2017). Comprehensive analysis of total protein content in soft wheat grain containing the *Gpc-B1* gene from *Triticum turgidum* ssp. *dicoccoides*. *Visnyk Ukrainskoho tovarystva henetykiv i selektsioneriv*, 1, 52—57 [in Ukrainian]. https://doi.org/10.7124/visnyk.utgis.15.1.712.
6. Lundström, M., Leino, M.W., Hagenblau, J. (2017). Evolutionary history of the *NAM-B1* gene in wild and domesticated tetraploid wheat. *BMC Genet*, 18, 118.
7. Nigarin, S., Shahidul, I., Juhasz, A., Ma, W. (2021). Wheat leaf senescence and its regulatory gene network. *The Crop Journal*, 9(4), 1—15. https://doi.org/10.1016/j.cj.2021.01.004.
8. Sengar, R. S., Singh, A. (2018). *Eco-friendly agro-biological techniques for enhancing crop productivity*. Singapore: Springer Nature.
9. Wani, S. H., Mohan, A., Singh, G. P. (2021). *Physiological, molecular, and genetic perspectives of wheat improvement*. Cham: Springer Nature Switzerland AG.
10. Chen, X., Song, G., Zhang, S., Li, Y., Jie, G., Shahidul, I., ..., Ji, W. (2017). The allelic distribution and variation analysis of the *NAM-B1* gene in Chinese wheat cultivars. *J. Integr. Agric*, 16, 1294—1303.
11. Morgun, V. V., Rybalka, O. I., Morgun, B. V. (2021). New scientific directions of genetic improvement of cereal crops. *Fiziolohiya roslyn i henetyka*, 53(3), 187—215 [in Ukrainian]. https://doi.org/10.15407/frg2021.03.187
12. Tabbita, F., Pearce, S., Barneix, A. (2017). Breeding for increased grain protein and micronutrient content in wheat: Ten years of the *Gpc-B1* gene. *J. Cereal Sci*., 73, 183—191. https://doi.org/10.1016/j.jcs.2017.01.003
13. Distelfeld, A., Uauy, C., Fahima, T., Dubcovsky, J. (2006). Physical map of the wheat high-grain protein content gene *Gpc-B1* and development of a high-throughput molecular marker. *New Phytol*, 169, 753—763.
14. Uauy, C., Distelfeld, A., Fahima, T., Blechl, A., Dubcovsky, J. (2006). A NAC gene regulating senescence improves grain protein, zinc and iron content in wheat. *Science*, 314, 1298—1301.
15. Venegas, J. P., Graybosh, R. A., Wienhold, B., Rose, D. J., Waters, B. M., Baenziger, P. S., ..., Amand, P. St. (2018). Biofortification of hard red winter wheat by genes conditioning low phytate and high grain protein concentration. *Crop Science*, 58, 1942—1953. https://doi.org/ 10.2135/cropsci2018.03.0175
16. Rybalka, O. I., Morgun, V. V., Morgun, B. V., Polishchuk, S. S. (2019). Genetic bases of the new direction of selection of wheat (*Triticum aestivum* L.) and triticale (*Triticosecale* Wittmack) classes that are original in terms of grain quality. *Fiziolohiya roslyn i henetyka*, 51(3), 207—240 [in Ukrainian]. https://doi.org/10.15407/frg2019.03.207.
17. Pokhylko, S. Yu. (2018). *Technological aspects of biofortification of soft wheat with the Gpc-B1 gene from Triticum turgidum ssp. dicoccoides*. (PhD) (Techn.). Kyiv [in Ukrainian].

18. Singh, C., Srivastava, P., Sharma, A., Kaur, H., Sohu, V. S., Bains, N. S. (2019). *Gpc-B1* mediated grain protein enhancement in wheat is compatible with high grain protein weight at moderated yield thresholds. *Indian J. Genet.*, 79 (2), 494—498. https://doi.org/10.31742/IJGPB.79.2.14.
19. Classifier of quality indicators of botanical taxa, the varieties of which undergo examination for suitability for distribution. (2019). Vinnytsia: Ltd "Tvory" [in Ukrainian].
20. *Patent of Ukraine № 65644*. Rybalka, O. I., Pokoievoi H. V. Device for determination of sedimentation sds-30 [in Ukrainian].
21. Khan, I. A., Procunier, J. D., Humphreys, G. D. (2000). Development of PCR-based markers for a high grain protein content gene from *Triticum turgidum* ssp. *dicoccoides* transferred to bread wheat. *Crop Science*, 40, 518—524.
22. Distelfeld, A., Uauy, C., Fahima, T., Dubcovsky, J. (2006). Physical map of the wheat high-grain protein content gene *Gpc-B1* and development of a high-throughput molecular marker. *New Phytologist*, 169, 753—763.
23. Kim, Y., Kim, D., Shim, D. (2008). Expression of the novel wheat gene TM20 confers enhanced cadmium tolerance to bakers' yeast. *Journal Biological Chemistry*, 283, 15893—15902. https://doi.org/10.1074/jbc.M708947200

Received 27.04.2023
Revised 04.06.2023
Accepted 05.06.2023

Б.В. Моргун[1, 2] (https://orcid.org/ 0000-0001-7041-6894/),
Н.В. Сандецька[1] (https://orcid.org/ 0000-0002-0558-2295/),
Л.Г. Великожон[1, 2] (https://orcid.org/ 0000-0002-5935-9363/)

[1] Інститут фізіології рослин і генетики Національної академії наук України,
вул. Васильківська, 31/17, Київ, 03022, Україна,
+380 44 257 5160, plant@ifrg.kiev.ua
[2] Інститут клітинної біології та генетичної інженерії Національної академії наук України,
вул. Академіка Заболотного, 148, Київ, 03143, Україна,
+380 44 526 7104, molgen@icbge.org.ua

ДІЯ ГЕНА *Gpc-B1* НА ВМІСТ ПРОТЕЇНУ В ЗЕРНІ ПШЕНИЦІ М'ЯКОЇ ОЗИМОЇ НА ТЛІ ГЕНЕТИЧНОГО ОТОЧЕННЯ УКРАЇНСЬКИХ СОРТІВ

Вступ. Нагальною проблемою селекції пшениці є питання покращення якості зерна. Одним із напрямків генетичного поліпшення пшениці є перенесення генів від диких родичів. Особливий інтерес у цьому напрямку становить ген *Gpc-B1*.

Проблематика. Використання сучасних систем ДНК-маркерів для визначення гена *Gpc-B1* від *Triticum turgidum* ssp. *dicoccoides* дасть можливість за короткий термін створити нові перспективні сорти-інновації з підвищеним вмістом протеїну у поєднанні з високими господарсько-цінними ознаками.

Мета. Визначити ефективність дії гена *Gpc-B1* у генетичному оточенні українського сорту 'Куяльник' на вміст протеїну в зерні, урожайність та хлібопекарські характеристики.

Матеріали й методи. Виявлення гена *Gpc-B1* проводили методом мультиплексної полімеразної ланцюгової реакції. Джерело гена *Gpc-B1* лінію Glu-Pro було схрещено з сортом 'Куяльник' (*Gpc-B1* × Куяльник). Вміст протеїну в зерні вимірювали методом інфрачервоної спектрометрії (NIR); визначення індексу седиментації за методом SDS-30 здійснювали на автоматичному приладі.

Результати. Розроблено домінантну та кодомінантну молекулярно-генетичні системи ДНК-маркерів для виявлення гена *Gpc-B1* від *T. turgidum* ssp. *dicoccoides* у лініях пшениці м'якої озимої. Аналіз зерна рослин поколінь F_5—F_9 показав, що ген *Gpc-B1* зумовлює підвищення вмісту протеїну на 3 % порівняно з вихідним сортом 'Куяльник'. Проаналізовано врожайність ліній носіїв гена *Gpc-B1* у польових умовах.

Висновки. Показано, що вплив гена *Gpc-B1* на урожай зерна практично відсутній на тлі генетичного оточення районованого українського сорту 'Куяльник'. Досліджено вплив цього гена на хлібопекарські характеристики пшениці м'якої. Лінії з геном *Gpc-B1* і без нього мали майже однаковий показник седиментації. Створено новітній селекційний матеріал пшениці м'якої озимої з підвищеним вмістом протеїну у зерні у поєднанні з високими господарсько-цінними ознаками, який готовий до передавання на Державне сортовипробування.

Ключові слова: Triticum aestivum L., ген *Gpc-B1, Triticum turgidum* ssp. *dicoccoides,* молекулярні маркери, вміст протеїну в зерні, показник седиментації.