

Identification of prospective sources of agronomically-valuable traits of bread wheat (*Triticum aestivum* L.) among breeding lines in the condition of Forest-Steppe of Ukraine

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Received: 06.10.2020. Accepted 06.11.2020

The aim of work was the estimation of valuable traits of bread wheat breeding lines, obtained from interspecies crosses with wild *Aegilops* and *Triticum* species growing in a condition of the Forest-Steppe of Ukraine. We used the seed proteins electrophoresis in PAAG for confirmation of the presence of rye seed storage components in the wheat parental lines genomes. The biochemical compositions of seeds had determined by the infrared spectroscopy method. As a result of researching from the set of 600 breeding lines were selected best lines with increased grain yield from 1 m², with high protein content in grain, disease resistance, and winter hardiness significantly exceeded the standard variety Poleskaya-90. All lines have high and moderate resistance against diseases: Powdery Mildew, Brown Rust, Septoria Blotch. Based on obtained data had selected breeding lines, which were promising sources of single and complex agronomically valuable traits for bread wheat breeding and genetic researches.

Keywords: Interspecific hybridization; Plant breeding; Goat grass; Bread wheat

Introduction

Wheat is one of the most important crops, the study of which is of great practical interest. The gene pool of the *Triticum aestivum* L. species is the object of intensive breeding work and versatile scientific research (Baenziger, 2009). Along with the progressive development of modern wheat breeding, the genetic diversity of the crop is gradually being exhausted, and it becomes more and more difficult for the breeder to find the desired combinations of genes over time (Rejesus, 1996). Among the known methods of extending the genetic diversity of bread wheat, interspecific hybridization took a leading place, since wild species of wheat characterized by a considerable genetic polymorphism of potentially value traits for wheat breeding (Prohens, 2017). Wild-growing species-relatives of wheat have always been in the field of view of breeders and geneticists, as donors of agronomically valuable traits for improving wheat. Especially noticeable are the achievements in the use of alien gene plasma in improving wheat varieties for signs of diseases and pests resistance (Ceoloni et al. 2017; Motsnyi et al. 2019; Devi et al. 2019; Hao et al. 2019; Sehgal et al. 2015; Kishii, 2019). Probable sources of economically valuable traits can be considered close species of the genus *Triticum*: *T. durum* Desf. (AABB genome), *T. timopheevii* Zhuk. (AtAtGG genome), *T. monococcum* L. (genome Am), *T. dicoccum* Schrank Schuebl. (AA genome), *T. spelta* L. (AABBDD genome), *Secale cereale* L. (DD genome), and *Aegilops*: *Ae. juveniles* (Thell.) Eig (genome DMU), *Ae. trincialis* L. (genome UC), *Ae. columnaris* Zhuk. (UM genome), *Ae. triaristata* Willd (genome UM), *Ae. cylindrica* Host (CD genome).

The gene pool of the genus *Aegilops* contains sources of resistance genes against several diseases: Brown Leaf Rust (causal agent – *Puccinia triticina* f. sp. tritici), Yellow Rust (causal agent – *P. striiformis* f. sp. tritici), Stem Rust (causal agent – *P. graminis* f. sp. tritici), Powdery mildew (causal agent – *Blumeria graminis* (DC.) Speer), Septoria Leaf Blotch (causal agent – *Septoria tritici*), Cercosporellosis (causal agent – *Cercospora herpotrichoides* Fron.), Head Smut (causal agents – *Tilletia laevis* (Kühn), *T. tritici* (Bjerk)) and loose smut (causal agent – *Ustilago tritici* (Pers.) C. N. Jensen, Kellerm. & Swingle), Helminthosporiosis (*Pyrenophora tritici-repentis* (Died) Shoem), Brown Leaf Spot (perenosporosis), Snow Mold, Root Rot, Barley Yellow Dwarf Virus, as well as some pests species (Cox et al. 1992; Chuang et al. 2017; King et al. 2018; Majka et al. 2018).

Some authors have convincingly proved that high winter hardiness of goatgrass (*Aegilops*) is associated with the D genome, whose carriers obviously in the course of evolution introduced this property to the bread wheat (Barashkova, 1984; Sutka, 1991; Palilova, 1991; Bulavka, 1989). The natural way of origin and occurrence of hexaploid wheat focused the attention of scientists on the

artificial synthesis of the hybrids of bread wheat and wild species. With this aim were investigated the breeding lines of wheat obtained of interspecific crossings by the characteristic of the range of traits. Wild cereals had been used as breeding sources of valuable traits of wheat in interspecific hybridization. The crossing of the locally adopted bread wheat varieties with wild cereal species is efficient in expanding the variability of traits, and increasing adaptability of breeding material is the perspective direction in wheat breeding.

The purpose of the study is to establish the breeding value of the source material of bread wheat created by interspecific hybridization, and to identify the most promising samples for individual economic and valuable characteristics and their combination for use in the breeding process.

Materials and Methods

The source material was created by interspecific crosses using biotechnological methods in the Department of Biotechnology of the Breeding Process of the V.M. Remeslo Myronivka Institute of Wheat of NAAS. Plants were grown in the field and greenhouses, hybridization carried out by the method of the emasculation of the single ear on intact plants, and the backcrossing carried out by the substitution method (Voloschuk, 2017). The source material tested in the experimental plots of the National Scientific Center "Institute of Agriculture of NAAS" located in the village of Chabany, Kyiv-Svyatoshynskiy district of Kyiv region (Forest-Steppe zone of Ukraine). In crossings took part 9 genotypes of bread winter wheat *Triticum aestivum* L. (Myronovskaya-61, Podolianka, Monotyp, Voloshkova, Bohdana, Odessa napivkarlykova, Balkan, Ca 8055, Lutescens 14662), 5 species of *Aegilops* spp: (*Ae. juvenalis* (K-681) *Ae. triuncialis* L. (K-1809), *Ae. columnaris* Zhuk. (K-1495), *Ae. triaristata* Willd (K-1435), *Ae. cylindrica* Host. (K-1810). During 2013-2014 were investigated 600 introgressive lines obtained by interspecific hybridization. Some parental forms of wheat varieties have the wheat-rye translocation, which has a positive influence on productivity. The standard variety for comparison was local breeding variety Polesskaya-90, which, by a combination of adaptability and many valuable traits, is close to the model of the varieties adapted to the Forest-Steppe zone.

Samples sowed in three-line plots of 1 m² with a row spacing of 15 cm. In the field were carried out observations and records according to state variety testing of crops (Babayants, 1998). The type, degree, intensity of development, and resistance to diseases were determined visually in the conditions of artificial infectious and natural provocative backgrounds, according to the methodology of phytopathological research (Motsnyii et al. 2019). The analysis of seed quality indicators was determined by infrared spectroscopy on the device *Infratec* 1241 (FOSS, Denmark) (Sozinov, 1997). The obtained indicators of productivity and quality of breeding samples were studied and compared using the methods of variation statistics. It identified the valuable indicators of the yield structure – length of a spike, number of spikelets per ear, number of grains, grain weight of ear, and mass of 1000 grains conducted by structural analysis. For a more objective selection of breeding samples, the fullness ear index used – the relation of the ratio of the number of grains in the ear to its length.

Storage proteins extracted and fractionated in the Laboratory of Environmental Genetics and Biotechnology of the Institute of Plant Protection of the NAAS. It used the method of electrophoresis of gliadins in polyacrylamide gel for determining wheat-rye translocation (Sozinov, 1997). Gliadin electrophoresis was performed in an acidic medium in a polyacrylamide gel (Kozub, 2001). The gliadin allele Gli-B11 (Gli-B1-3) was a genetic marker of wheat-rye 1BL/1RS translocation (Kozub, 2009). The Gli-B11 allele was identified by the catalog (Metakovsky, 1991). The bread wheat variety Bezostaya-1 had used as a reference spectrum for allelic identification.

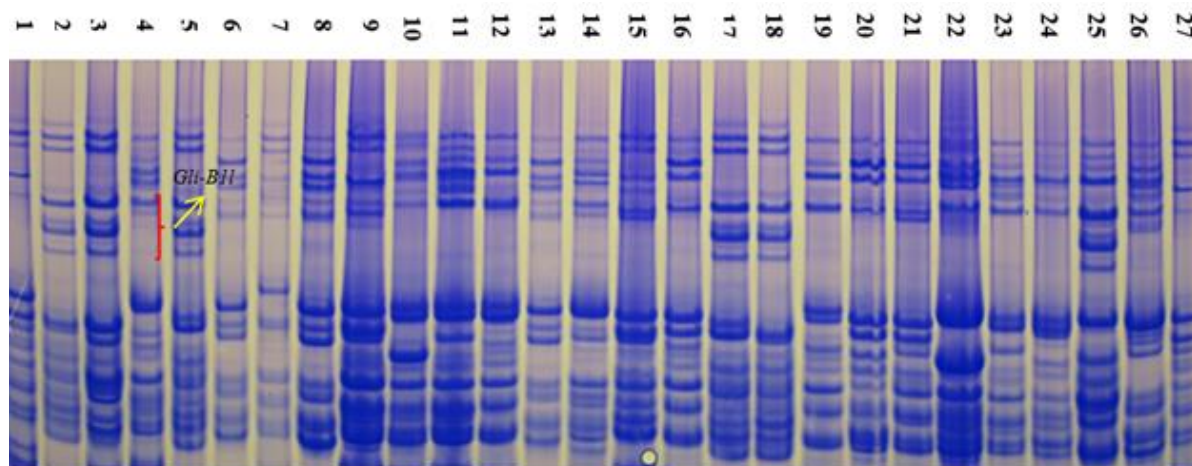


Figure 1. Electrophoregram of gliadin grains of the offspring of interspecific crossings in PAGE: 1) – Bezostaya-1 (etalon spectrum); 2) – line 2567; 3) – line 2566; 4) – line 2511; 5) – line 2526; 6) – line 2485; 7) – line 2527; 8) – line 2484; 9) – line 2508; 10) – line 2521; 11) – line 2520; 12) – line 2503; 13) – line 2480; 14) – line 2486; 15) – line 2518; 16) – line 2310; 17) – line 2500; 18) – line 9; 19) – line 6; 20) – line 2480; 21) – line 2565; 22) – line 2487; 23) – line 2537; 24) – line 2481; 25) – line 2488; 26) – line 2492; 27) – Bezostaya-1.

Results

Considerable attention was to genotypes carried wheat-rye 1BL/1RS translocation, which is a marker of multiple-locus of resistance against rust diseases Sr31/Lr26/Yr9 and is probably associated with increased adaptability and productivity (Kozub, 2001). Such genotypes can combine the value of disease resistance and productivity. As a result of the electrophoresis of storage proteins, six samples with wheat-rye translocation identified (Figure 1). It indicates the possibility of combining the wheat genome of rye components and the genetic material of other distant relatives. For identifying other introgressions of alleles of storage proteins on gels, it is necessary to perform an analysis of hybrids and compare the spectra of parent forms. Rye components were most likely obtained from the original wheat varieties that were used in hybridization (Mironovskaya-61, Podolyanka, Monotype, Voloshkova, Bogdana, Odessa napivkarlykova, Balkan, Ca-8055, Lutescens-14662) and have a complex of valuable traits: productivity,

adaptability, disease resistance, winter hardiness. These genotypes are not optimal for baking properties, but their flour can be used in a mixture with the flour of high-quality wheat.

All breeding lines analyzed by the different agronomy-valuable traits like yielding, yield structure components, the height of the plant, quality indices, and disease resistance. The yield of the best-selected quality lines (Table 1) ranged from 602.1 to 785.6 g per 1 m². The average yield of breeding lines was 656.5 g / m². The highest grain yield from 1 m² obtained at the line 2520/2 (785.6 g/m²), which is significantly higher than the standard variety Polesskaya-90 (634.1 g/m²). However, in terms of the mass of 1000 grains, this line was significantly lower to the standard by 5.6%. The average mass of 1000 grains among the samples varied from 30.1 to 46.6 g. The line 72/1 with a weight of 1000 grains 46.6 g was more than the standard variety on the high significance level. The height of the plant among the numbers varied from 110 – 144.6 cm, close to the parameters of the semi-dwarf variety. At the same time height of all samples exceeded the standard variety Polesskaya-90. There was no determination of the propensity to lodging, but individual genotypes had a durable filled straw, which probably could have a positive effect on this trait.

The lines also differed on the yield structure components. The results of measurements of the ear length of breeding lines indicate a significant variability of this trait in the investigated set of wheat breeding samples. The length of the ear varied from 6.5-9.6 cm, and the average was 7.8 cm. All lines significantly exceeded the standard for the length of the ear, while on the number of spikelets in the ear were better only in breeding lines 2440/1, 2661/2, 72/1, 72/2, 2516, 2521/1, and 2494/1. The spikelet number per head varied from 12 to 18 pcs per ear, and the number of seeds per head ranged from 31 to 39 pieces. The highest index was in the line of 2516 (5.23), which was higher than the standard variety for this indicator and had a dense and well-grained ear. However, sample 2516 has the average weight of grain from the ear was at the level of the Polesskaya-90 (1.44 g), which was 1.42 g. This indicator was significantly high in line 2440/1 (1.58 g), so it probably is considered as a valuable source of high average grain weight from the ear.

Table 1. The characteristic of yield structure components of best bread wheat introgressed breeding lines, 2013 – 2014.

Breeding name/number	line	Yield per 1 m ² , g/m ²	St to Polesskaya-90	Weight of 1000 seeds, g	Plant height, cm	Length of ear, cm	Number of spikes, pcs.	Number of seeds per ear, pcs.	Fullness ear index	Weight of seeds per ear, g
St Polesskaya-90		634.1	-	45.0	110.0	6.5	14	32	4.92	1.44
2440/1		631.2	-2,90	43.1	136.8	8.3	18	37	4.41	1.58
2661/2		657.3	23,20	30.1	129.0	9.6	18	39	4.10	1.19
72/1		623.5	-10,60	46.6	144.6	7.9	15	32	3.90	1.44
72/2		694.7	60,60	42.9	139.0	8.4	16	32	3.76	1.36
2558		618.2	-15,90	34.2	120.4	6.9	14	32	4.67	1.10
2516		602.1	-32,00	36.2	135.0	7.5	16	39	5.23	1.42
2521/1		695.3	61,20	39.1	126.0	7.4	15	29	3.95	1.14
2520/2		785.6	151,50	39.4	128.2	7.8	14	28	3.59	1.10
2494/1		652.7	18,60	36.0	138.6	8.4	16	32	3.83	1.16
2488		627.2	-6,90	36.0	140.8	6.9	12	28	4.09	1.02
Average		656.5	-	39.0	131.7	7.8	15	33	-	1.30
Min		618.2	-	30.1	110.0	6.5	12	31	-	1.02
Max		785.6	-	46.6	144.6	9.6	18	39	-	1.58
Sx		10.9	-	1.1	2.10	0.2	0.3	0.8	-	0.0
V%		7.9	-	12.9	7.7	11.3	10.8	12.3	-	14.5
S		52.12	-	5.04	10.19	0.88	1.63	4.03	-	0.18
LSD05		31.8	-	3.1	6.2	0.5	1.0	2.5	-	0.10

Notes: St – Standard Variety, Sx – Average Sample Size, S – Standard Deviation, V% – Coefficient of Variation, LSD – Less Significant Difference, Min - Minimum Value, Max – Maximum Value

Also were studied the biochemical parameters of obtained hybrid descendants. Table 2 shows the characteristics of the top 10 lines with a protein content of 18.0-20.4% in the grain, which significantly exceeded the standard variety Polesskaya-90 with a protein content of 13.1% by 4.9-7.3% (LSD05=1.1). The gluten content in the selected lines was at the level of 31.3-34.7%, which is significantly higher by 8.1-11.5% (LSD05=1.8) compared to the standard (23.2%). The Zeleny index was 70.6-80.6%, which significantly exceeds the standard variety (47.1%) by 23.5-33.5% (LSD05=5.3). The coefficient of variation of the sample was 9.8, 9.3 and 12.1%, respectively. The correlation analysis of these indicators confirmed a close correlation between the content of protein, gluten, and Zeleny number in the grain ($r=0.98 - 0.99$), as well as a close inverse correlation between the starch and protein content, gluten, and Zeleny index in the grain ($r=-0.94 - -0.95$). Were determined that winter wheat lines with a high content of protein, gluten and Zeleny sedimentation index respectively have a low starch content. All lines had the resistance to diseases (Powdery Mildew, Brown Rust, Septoria Leaf Blotch) from 7 points (the lesion estimated as less than 20%) to 9 points (there are no signs of damage). As a result of the biochemical analysis revealed a weak correlation between yield from 1 m² and grain protein content, gluten, and Zeleny index, which manifested in the tendency ($r=0,04 - 0,11$); and the inverse relationship between the mass of 1000 seeds and content in grain protein, gluten and Zeleny index ($r=- 0,04 - -0,45$).

The vast majority of the lines characterized by high winter hardiness, the average score was 9. The height of the plant of breeding lines was higher than the standard variety Polesskaya-90 and averaged 131.7 cm. A close correlation finds between the height of plants and the content of protein, gluten in the grain, and the Zeleny index ($r=0.71 - 0.72$). However, there is also an inverse strong correlation between plant height and grain starch content ($r=- 0.73$).

Given the results of the research, the selected introgressive lines are valuable breeding material, which we can use as sources that have a set of positive characteristics. Therefore, evaluating each line, we have identified several that are appropriate to use as sources of economically valuable features. Line 72/2 with a high content of protein in the grain (18.5%), gluten (32.3%), Zeleny (74.4%), with an ear length of 8.4 cm, the number of spikelets – 15.8 pcs., with high resistance to Powdery Mildew (9 points), Brown Rust (9 points), Septoria (8 points); with winter hardiness 9 points. Line 2516 also had a high content of protein in the grain

(18.7%) and other characteristics: gluten (33.4%), Zeleni index (77.4%), ear length (7.5 cm), number of spikelets (16 pcs.), number of grains in the ear – 39.2 pcs., resistance against powdery mildew 9 points, brown rust resistance– 9 points, Septoria leaf blotch resistance – 8 points; high winter hardiness 8 points. Sample "2494/1" had a low content of protein in the grain 18.1%, low gluten content - 31.9%, Zeleny index 74.6%, and other traits: ear length 8.4 cm, number of spikelets in the ear 15.6 pcs., resistance to powdery mildew (9 points), brown rust resistance (9 points), Septoria Leaf Blotch resistance (8 points), winter hardiness (9 points).

Table 2. Seed quality characteristics and disease resistance of the best lines of introgression breeding lines, 2013 – 2014 (average).

Breeding line name/number	Seed quality					Disease resistance, points				
	Protein, %	± St to Poleskaya-90	Starch, %	Gluten, %	± St to Poleskaya-90	Index Zeleny	Powdery Mildew	Brown Rust	Septoria Leaf Blotch	Winter hardiness
Poleskaya-90 (St)	13.1	-	67.2	23.2	-	47.1	7	7	6	8
2440/1	18.3	5.2	60.9	31.3	8.1	72.6	9	8	8	9
2661/2	18.0	4.9	62.4	31.6	8.4	72.0	9	8	8	9
72/1	18.2	5.1	60.6	31.6	8.4	70.6	9	8	8	8
72/2	18.5	5.4	60.3	32.3	9.1	74.4	9	9	8	9
2558	18.4	5.3	60.6	31.9	8.7	71.9	8	8	8	8
2516	18.7	5.6	60.3	33.4	10.2	77.4	9	9	8	8
2521/1	18.6	5.5	59.9	32.9	9.7	76.1	9	8	8	9
2520/2	18.2	5.1	59.7	31.5	8.3	73.6	8	8	8	9
2494/1	18.1	5.0	59.3	31.9	8.7	74.6	9	9	8	9
2488	20.4	7.3	57.7	34.7	11.5	80.6	8	8	7	9
Average	18.0	-	60.8	31.5	-	71.9	-	-	-	-
Min	18.0	-	57.7	31.3	8.1	70.6	-	-	-	-
Max	20.4	-	62.4	34.7	11.5	80.6	-	-	-	-
Sx	0.4	-	0.5	0.6	-	1.8	-	-	-	-
V%	9.8	-	4.0	9.3	-	12.1	-	-	-	-
S	1.77	-	2.41	2.93	-	8.70	-	-	-	-
LSD05	1.1	-	1.5	1.8	-	5.3	-	-	-	-

Notes: St – Standard Variety, Sx – Average Sample Size, S – Standard Deviation, V% – Coefficient of Variation, LSD – Less Significant Difference, Min - Minimum Value, Max – Maximum Value.

Discussion

Interspecies hybridization of the bread wheat with wild cereal species successfully used in breeding for disease resistance. Interspecies hybridization use solve several tasks simultaneously: transfer in genotype recipient new coadaptive clusters of genes formed in the process of interaction of species with the environmental condition, widening of the variability of quality and quantity traits, the formation of new combinations of genes, induction of interspecies heterosis. One of the factors inflated on the amount of valuable initial breeding material obtained by interspecies hybridization is the growing climatic condition and type of the selected traits. Hybrids grew in the Forest-Steppe zone of Ukraine, which was characterized by the temperate climate and severe winters in some years. There were selected distinct traits and complex traits. The helpful instrument of implementation in interspecies breeding is molecular markers. The scientists from Odessa have successfully crossed the bread wheat with *Aegilops cylindrica*. Obtained hybrid descendants were showed resistance against head smut (*Tilletia caries*) (Galaev, 2003; Galaev, 2006). The transfer of disease resistance genes from wild grasses is one of the widely used perspective methods in wheat applied genetics and breeding process (Antonyuk, 1998). Ukrainian scientists used the molecular markers in the identification of introgressive genetic material in substitution lines of bread wheat (Antonyuk, 2001). With this purpose were crossed as a tetraploid component bread wheat variety Aurora and diploid species of goat grass: *Ae. umbellulata*, *Ae. mutica*, *Ae. sharonensis*, *Ae. speltoides*, *Ae. uniaristata* and developed microsatellite markers specific for chromosomes 5A, 5B, 5D for identification of alien introgression from the genome of *Aegilops mutica* (T.) (Zhirov, 984; Yephymenko, 2014).

Ukrainian scientists were developed breeding lines with an exotic cluster of gliadins and glutenins *Gli-D1cy1/Glu-D1cy1* determined by the SDS-electrophoresis technics, introgressed in common wheat from local species of *Aegilops cylindrica* on a basis of interspecies hybrids of Doctor O.I. Rybalko. On the information given by the authors, these genotypes can be a valuable source of high-quality wheat flour (Rybalka, 1993; Morgun, 2014).

In the same way, developed new genetic material obtained by the *T. kiharae* i *T. miguschovae* with bread wheat crosses with protein markers and ISSR-markers use. In genotypes of hybrids of bread, wheat was determined the introgression from *T. kiharae* by the *Gli-A1*, *Gli-D1*, *Glu-D1* locus, and in some hybrids identified the introgression of the locus *Glu-G1* from *T. miguschovae* (Tverdokhlebl, 2011). It proves the opportunity of the *T. kiharae* i *T. miguschovae* usage in developing of the new wheat breeding material (Tverdokhlebl, 2011).

The rye (*Secale cereale* L.) also has a long history of usage as a source of resistance against diseases, ecological plasticity, and high productivity for wheat [37, 38]. The translocation from R-genome in wheat, in some cases, has a positive effect on multiple traits but negative on bread-making quality (Audenaert, 2014; Willenborg, 2008). In the last researching of storage proteins of Ukrainian

bread wheat varieties of different years of developing were detected high frequencies of wheat-rye translocation in genotypes, which can indicate the possible connection of it with resistance to abiotic stress and productivity.

Similar investigations of the initial material of bread wheat and wild cereals crosses is an example of the successful transfer of some agriculture-valuable traits in the wheat genome and opportunity of its usage with the aim of genetic improvement in Ukraine's climatic conditions. The disadvantage of such crosses is, as a rule, the displacement of genome parts and gene clusters that harm genotype yield and grain quality traits. Such signs include low threshing ability, thick hulls, loss of 1000 seeds weight, etc.

In the results of our studies of a set of 600 breeding samples (obtained from interspecies crosses), only 10 (1.7%) were promising as a source of several agronomically valuable traits. But in combination with other breeding methods (mutagenesis, back crossings, biotechnology technologies, marker-assisted selection) the effectivity of selection process can be sharply increased.

Conclusion

Researching of 600 breeding lines from interspecies crosses of 9 genotypes of winter bread wheat (*Triticum aestivum* L.) varieties as a parent component with 5 species of *Aegilops*, and the wild species of *Triticum* (*T. timopheevii*, *T. monococcum*, *T. dicoccum*, *T. spelta*) were selected 10 lines (1.7% of the total number) which have high productivity (618.2 – 785.6 g/m²) and yield structure parameters, the favorable height of plant (110 – 144.6 cm), increased protein content in the grain (18.0 – 20.4%), high gluten content (31.3 – 34.7%), Zeleny index (71.9 – 80.6) and winter hardiness, significantly exceeded the standard variety Polesskaya-90. The highest grain yield of 1 m² was found at the line 2520/2 (785.6 g), which is significantly higher than the standard variety Polesskaya-90 with a grain yield of 634.1 g/m². All lines characterized by high and moderate resistance to leaf diseases (Powdery Mildew, Brown Rust, Septoria Leaf Blotch) from 7 points (the lesion was estimated as less than 20%) to 9 points (there are no signs of damage). Introgressive lines 72/2, 2516, and 2494/1 are promising sources of a complex of agronomically valuable traits, and also can be used in wheat breeding and genetic researches.

Funding

This study was performed and funded under the state research program 11 "Cereal crops" of National Academy of Agrarian Sciences of Ukraine.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

S.K. designed and performed the field experiments, performed the yield structure and seeds biochemical analysis, derived the results, analyzed the data and drafted article; S.V. developed the introgression breeding material and designed the experiment strategy; N.K., performed the storage proteins electrophoresis, visualization and provided critical revision of the article. Y.Z. performed the sample preparation, extraction, purifying of storage proteins, seeds biochemical analysis and co-wrote the paper. V.S. supervised the research and translates the paper.

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Citation:

Zaika, Ye.V., Kovalchuk, S.O., Voloschuk, S.I., Kozub, N.A., Starychenko, V.M. (2020). Identification of prospective sources of agronomically-valuable traits of bread wheat (*Triticum aestivum* L.) among breeding lines in the condition of Forest-Steppe of Ukraine. *Ukrainian Journal of Ecology*, 10(5), 253-258.



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