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## USE OF SPELT WHEAT (*Triticum spelta* L.) IN BREEDING TRITICALE (*Triticosecale* Wittmack) FOR GRAIN QUALITY

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## Abstract

Poor grain quality, mostly low proteins and gluten content, together with worse gluten quality, are the main obstacle to the widespread practical use of triticale. Hybridization of hexaploid triticale with certain *Triticum* species significantly expands the crop gene pool diversity and facilitates production of new forms with improved quality indicators. Spelt wheat is much suitable for crossing due to high protein content in grain (up to 25 %). The first Ukrainian study on hybridization between hexaploid triticale and spelt wheat was carried out in the Uman National University of Horticulture. The aim of the research was to improve triticale grain by intergeneric hybridization with spelt wheat and characterization of the hybrids for grain quality. The crosses resulted in a collection of more than 500 breeding samples of triticale which were grouped by plant height as medium, dwarf and short-stem forms. The best samples were analyzed for grain quality, i.e. content of proteins and gluten, the gluten quality, the 1000-seed weight and grain unite. Winter triticale Rarytet cultivar was the standard for medium height samples, and Alkid cultivar for low and short stem samples. Our research shows the improvement of triticale grain quality by intergeneric hybridization with spelt. Genotypes No. 455, 468 and 475 of medium height group, together with all dwarf and short-stem samples significantly exceeded the standards in grain protein and gluten content. The samples Nos. 455 (13.9 % protein, 30.2% gluten), 468 (13.0 % protein, 27.1 % gluten) and 473 (12.8 % protein, 28.0 % gluten) outstood for grain protein and gluten concentration. By the set of gluten quality indicators, the samples Nos. 455, 458, 451, 466, 488, 471 and 473 were assigned to group I, the rest of the samples belonged to group II. The samples Nos. 455 (56.0 g), 471 (55.3 g) and 473 (54.7 g) significantly surpass the standard in 1000-seed weight. No significant differences were found between the samples within each group for grain unit values. The highest values have Nos. 455 (700 g/l), 471 (690 g/l), 469 and 473 (685 g/l), 484 (682 g/l). As a result, two genotypes with high indicators of grain quality were selected, i.e. medium height sample No. 455 with protein content 13.9 %, group I gluten 30.2 %, 1000seed weight 56.0 g, grain unit 700 g/l, and short-stem sample No. 471 with protein content 13.6 %, group I gluten 29.5 %, 1000-seed weight 55.3 g, and grain unit value 690 g/l).

Keywords: Triticosecale Wittmack, hexaploid triticale, Triticum spelta L., spelt wheat, hybridization, protein content, gluten content, 1000-seed weight, grain unite

Hexaploid triticale (*Triticosecale* Wittmack) is grown as a forage, food, and technical crop. Its planting acreage reaches 4 million ha [1, 2]. The priority directions of triticale selection are the increase in productivity, stability, and the improvement of grain quality indicators [2-5]. Low quality indicators of triticale grain, in particular, low content of protein and gluten in the grain, as well as low quality of gluten, are the main obstacles to the widespread introduction of the crop into production [6, 7]. Therefore, the efforts of many breeders are aimed at improving the quality of grain crops [8-10]. The insufficiently high plasticity of

cultivars and selective forms of triticale is associated with a limited genetic diversity of the parental material; it is required to expand the gene pool and increase the effectiveness of its selection with various methods, in particular, hybridization of hexaploid triticale with the genus *Triticum* L. species [11, 12]. As is noted by Hills et al. [13], it is an efficient way to increase the genetic diversity of the crop significantly. It is advisable to use spelt wheat (*Triticum spelta* L.), a hexaploid species ( $2n = 6 \times = 42$ ) with a genomic composition, homologous to the soft wheat (A<sup>u</sup>BD) for hybridization. Spelt wheat grain contains up to 25% protein [14, 15]. It has been shown [16, 17] that spelt wheat grain contains essential amino acids, which are not synthesized in soft wheat grain and cannot be obtained from foodstuff of animal origin.

The idea of combining the genetic material of hexaploid triticale with spelt wheat belongs to Parii [11, 18]. As a result of experiments on transfer of the spelt wheat genetic material during hybridization of hexaploid triticale with spelt, a genetic pool of the Uman National University of Horticulture was created which includes more than 500 samples. It consists of recombinant forms that differ in economically valuable and morpho-biological characteristics.

The present paper represents the analysis of the hexaploid triticale forms obtained after hybridization with spelt wheat (according to protein and gluten content, gluten quality, 1000 seed weight and grain unit) and proves that it is possible to improve the quality of triticale grain in this way.

The aim of the research was to improve triticale grain characteristics by intergeneric hybridization with spelt wheat and characterization of the hybrids for the main indicators of grain quality.

*Techniques.* The hybridization of two types of grain crops and stabilization of the received samples were carried out during 2006-2012 (Central foreststeppe of Ukraine, the experimental field of the Uman National University of Horticulture, the Cherkasy Region). In hybridization, hexaploid triticale (*Triticosecale* Wittmack) Rozovskaya 6, Rozovskaya 7, or Ladnaya cultivars were a female parent, and the winter spelt wheat (*Triticum spelta* L.) Zarya Ukrainy cultivar was a pollinator. Hybridization was carried out by castration of flowers of the female parent, followed by controlled pollination with the seed parent. Glutenin proteins were electrophoretically separated in PAAG according to the description [19].

Grain quality indicators of the obtained breeding samples were analyzed ( $F_{5-10}$ , 2012-2017). All phenological observations and tests were conducted in accordance with the State Methodology for Qualifying Examination of Plant Varieties with the Definition of the Suitability Indicators for Proliferation in Ukraine (Ukrainian Institute for Plant Variety Examination, Kiev, 2012, vol. 2). Samples of triticale were grouped according to plant height based on the classification by Shchipak [20]. The productivity of the samples was evaluated in 5-fold repetition on 10 m<sup>2</sup> plots placed systematically. Winter triticale Rarytet cultivar was a medium-height standard, and winter triticale Alkid cultivar was dwarf and short-stem standard.

Experimental data were analyzed statistically with Microsoft Excel 2010 software. The mean values (*M*) and standard errors of means ( $\pm$ SEM) were calculated. The least significant difference (LSD<sub>05</sub>) and the coefficient of variation (*Cv*) were calculated according to Ehrmantrout [21].

*Results.* The obtained  $F_1$  hybrids were characterized by uniformity in spike morphology and general habitus of plants. Plants have dominative characteristics of spelt wheat (a long loose spike, rough glume, awnlessness, and worsened threshability).

By hybridization of hexaploid triticale and spelt wheat, mainly sterile F<sub>1</sub>

plants appeared. Only a few samples formed fertile pollen grains. The sterility of plants obtained as a result of remote hybridization is highlighted by many authors [22, 23]. Thus, the  $F_1$  hybrids from hybridization of three-species triticale and soft wheat are sterile, as in the case of hybridization of triticale and spelt wheat. The reason is the fact that between the AB triticale and wheat genomes, normal bivalent conjugation takes place because these genomes are homologous. Chromosomes of triticale R and spelt wheat D genomes do not have pairs for conjugation in meiosis. The process of meiosis among them is accompanied by significant anomalies, chromosomes form univalents that do not conjugate with each other. This leads to the formation of aneuploid gametes resulting in aneuploid plants. The fertility of such plants is reduced sharply.

The studies by Pershina and Trubacheva [23] show that it is possible to increase the pollen fertility of sterile  $F_1$  hybrids by re-hybridizations with one of the parents. We also used this technique in this work and back-crossed the  $F_1$  hybrids with hexaploid triticale. The spelt wheat-associated traits were less noticeable among the back-crossed descendants. Plant variability on the phenotype varied in  $F_1BC_1$  hybrids and went beyond that of the parents.

Self-pollination of  $F_1BC_1$  hybrids for several generations was used to stabilize the samples. After each subsequent self-pollination, the percentage of stable and well-grained forms of triticale increased. After the fifth self-pollination, 1137 plants with spike grain content equal to the original form were identified, 316 of them were grained for more than 80%, 471 - for 71-80% and 350 - for 61-70%.

Electrophoretic analysis of grain gluten proteins in PAAG revealed samples with genetic material from spelt wheat that carry spelt-specific gliadin- and glutenin-coding loci in chromosomes of the first homeological group, the *Gli-B1*, *Gli-D1*, *Gli-B5*, *Gli-A3*, *Glu-A1*, *Glu-B1*, and *Glu-D1*.

The resulting triticale forms were divided into three groups by plant height based according to the Shchipak's classification [20]: medium-height (100-120 cm), dwarf (80-100 cm) and short-stem (60-80 cm). The best geno-types were selected in each group for further analysis of economically valuable indicators.

The main purpose of hybridization of hexaploid triticale with spelt wheat was the genetic improvement of triticale, the increase in grain protein and gluten content which would improve its baking and technological properties. Lukaszewski [7] and Ukalska et al. [24] showed that triticale grain contains 10-12% protein, 20-25% gluten and is significantly inferior to soft wheat grain. Other researchers came to the same conclusions [8, 10, 17]. At the same time, it is admitted that the crop improvement potential in terms of productivity and quality is quite high, and the possibilities of its application are diverse [25].

In this experiment, a weak variation in the protein content (Cv = 8.9%) and average in the gluten content (Cv = 11.4%) occurred in the medium-height group. Sample No. 455 exceeded the standard (Table) in the protein content significantly (by 2.2% at LSD<sub>05</sub> = 0.4). The same sample had gluten content (30.2%) significantly higher (by 5.7% at LSD<sub>05</sub> = 1.1) than that of the standard. Other analysed samples exceeded the standard by 0.5 to 9.8% (LSD<sub>05</sub> = 1.1). Samples No. 468 (13.0%), No. 475 (12.5%), and No. 458 (12.3%) of this group had high grain protein content. They also exceeded the standard cultivar in the gluten content (0.7-1.8% at LSD<sub>05</sub> = 1.1). Gluten quality similar to that in group I was in samples Nos. 455 and 458. Other medium-height forms as per the indicators of gluten quality belonged to group II, which is mainly due to the low gluten deformation index (GDI), the main indicator characterizing its quality. The 1000 seed weight had the largest variation range (Cv = 18.7%). Sample No. 455 (56.0 g) (LSD<sub>05</sub> = 2.1) exceeded the standard in this indicator significantly. It is necessary to note that sample No. 455 has the highest indicators of the protein and gluten content, 1000 seed weight, and test weight (see Table) in the experiment.

Selection sample	Protein, %	Gluten			1000-grain	Test
		content, %	GDI	quality group	weight, g	weight, g/l
Medium stem (100-120 cm)						
Raritet (St)	$11.7 \pm 0.04$	$25.5 \pm 0.11$	75	Ι	$50.4 \pm 0.24$	$670 \pm 12$
455	13.9±0.02	$30.2 \pm 0.06$	75	Ι	$56.0 \pm 0.32$	$700 \pm 8$
458	$12.3 \pm 0.03$	$26.2 \pm 0.07$	70	Ι	$48.9 \pm 0.20$	$660 \pm 14$
461	$11.5 \pm 0.04$	$24.1 \pm 0.05$	60	II	$48.4 \pm 0.21$	662±11
465	$11.5 \pm 0.05$	$24.6 \pm 0.07$	60	II	49.3±0.23	665±9
468	$13.0 \pm 0.03$	$27.1 \pm 0.08$	55	II	$50.2 \pm 0.25$	$670\pm5$
475	12.5±0.03	$27.3 \pm 0.09$	60	II	$51.4 \pm 0.27$	$678 \pm 8$
LSD <sub>05</sub>	0.4	1.1			2.1	28
Min	11.5	24.1			48.4	665
Max	13.9	30.2			56.0	700
Cv, %	8.9	11.4			18.7	10
S <sub>x</sub> , %	4.6	3.5			3.3	4.3
Dwarf stem (80-100 cm)						
Alkid (St)	$10.0 \pm 0.04$	$21.4 \pm 0.07$	45	II	$50.2 \pm 0.25$	$680 \pm 11$
451	$12.0 \pm 0.03$	$26.0 \pm 0.07$	65	Ι	$45.3 \pm 0.17$	$650 \pm 14$
467	$12.2 \pm 0.04$	26.8±0.09	50	II	49.6±0.21	665±9
484	$12.4 \pm 0.04$	$26.9 \pm 0.04$	60	II	$50.3 \pm 0.24$	682±11
486	$11.7 \pm 0.03$	$25.8 \pm 0.05$	70	Ι	$47.7 \pm 0.18$	657±15
488	$12.6 \pm 0.06$	$27.7 \pm 0.12$	65	Ι	$47.8 \pm 0.17$	660±12
LSD <sub>05</sub>	0.4	1.1			2.0	28
Min	10.0	21.4			45.3	650
Max	12.6	27.7			50.3	682
Cv, %	8.5	11.8			10.2	10
S <sub>x</sub> , %	4.1	3.7			3.0	4.0
			stem (60-			
Alkid (St)	$10.0 \pm 0.04$	$21.4 \pm 0.07$	45	II	$50.2 \pm 0.25$	$680 \pm 8$
469	$11.4 \pm 0.05$	$25.8 \pm 0.10$	50	II	$51.3 \pm 0.27$	685±6
470	$12.6 \pm 0.03$	$26.4 \pm 0.08$	70	Ι	49.7±0.22	$660 \pm 12$
471	$13.6 \pm 0.02$	$29.5 \pm 0.05$	70	Ι	$55.3 \pm 0.30$	690±7
473	$12.8 \pm 0.03$	$28.0 \pm 0.06$	65	Ι	54.7±0.29	685±6
468	$11.6 \pm 0.05$	26.1±0.09	60	II	$47.2 \pm 0.18$	655±11
LSD <sub>05</sub>	0.5	1.2			1.9	27
Min	10.0	21.4			47.2	655
Max	13.6	29.5			54.7	690
Cv, %	10.0	15.7			17.2	10
S <sub>x</sub> , %	4.2	3.8			3.5	3.8
N o t e. GDI – gluten deformation index, $S_x$ – test error.						

Quality indicators of grain among selection samples of *Triticosecale* Wittmack/*Triticum spelta* L. (*M*±SEM, Ukraine, the Cherkasy Region, 2012-2017)

Dwarf and short stem triticale cultivars are not used in agricultural production. As highlighted by Barnett et al. [26], this is due to the presence of a negative correlation between plant height and productivity. However, according to Kurkiev [27], such correlations are not absolute and manifested under adverse environmental conditions. Therefore, triticale forms which have high quality and productivity indicators combined with dwarf or short stems are of particular interest.

In these studies, all dwarf and short-stem forms were significantly superior to the standard in terms of the protein content (by 1.4-3.6% at LSD<sub>05</sub> = 0.4-0.5) and gluten content (by 4.4-8.1% at LSD<sub>05</sub> = 1.1-1.2) (see Table). The variation was average in gluten content (Cv = 11.8-15.7%), and low in grain protein content (Cv = 8.5-10.0%). The Alkyd cultivar was distinguished by high yield, but had low quality indicators: the protein content in the grain did not exceed 10.0% with 21.4% gluten. In the dwarf group, the increased protein (12.6 and 12.4\%) and gluten (27.7 and 26.9\%) contents were registered in samples Nos. 488 and 484, respectively. Samples Nos. 451, 486, and 488 showed group I gluten quality indicators. Sample No. 484 exceeded the standard cultivar for

1000 seed weight by 0.1 g, while samples Nos. 451, 467, 486, and 488 were inferior to it in this indicator. According to test weight, samples Nos. 484 (682 g/l), 467 (665 g/l), and 488 (660 g/l) (see Table) stood out.

Among the short stem forms, sample No. 471 was the best in the protein and gluten content, with 13.6% grain protein and 29.5% gluten, which was one of the highest indicators during the experiment. Samples No. 470 (12.6% protein, 26.4% gluten) and No. 473 (12.8% protein, 28.0% gluten) were slightly inferior. These samples differed from the rest due to combination of high 1000 seed weight, test weight, and gluten quality (group I).

Thus, these data evidence the possibility to improve triticale grain quality by intergeneric hybridization involving spelt wheat in breeding. In this work we obtained two hybrids with high grain quality indicators, i.e. the medium-height sample No. 455 (13.9% grain protein, 30.2% gluten of group I, 56.0 g 1000 seed weight, 700 g/l test weight) and short-stem sample No. 471 (13.6% protein, 29.5% gluten of group I, 55.3 g 1000 seed weight, 690 g/l test weight).

## REFERENCES

- 1. Estrada-Campuzano G., Slafer G.A., Miralles D.J. Differences in yield, biomass and their components between triticale and wheat grown under contrasting water and nitrogen environments. *Field Crops Research*, 2012, 128: 167-179 (doi: 10.1016/j.fcr.2012.01.003).
- 2. Grebennikova I.G., Aleinikov A.F., Stepochkin P.I. Vychislitel'nye tekhnologii, 2016, 1: 53-64 (in Russ.).
- 3. Rybalka O.I., Morgun V.V., Morgun B.V., Pochinok V.M. *Fiziologiya rastenii i genetika*, 2015, 2: 95-111 (in Ukr.).
- 4. Shchipak G.V. Selektsiya polevykh kul'tur, 2008, 5: 42-88 (in Ukr.).
- 5. Dennett A.L., Cooper K.V., Trethowan R.M. The genotypic and phenotypic interaction of wheat and rye storage proteins in primary triticale. *Euphytica*, 2013, 194: 235-242 (doi: 10.1007/s10681-013-0950-y).
- 6. Leshchenko N.I., Shakirzyanov A.Kh., Myzgaeva V.A., Karachurina G.R. *Dostizheniya nauki i tekhniki APK*, 2010, 1: 16-19 (in Russ.).
- Lukaszewski A.J. Cytogenetically engineered rye chromosomes 1R to improve bread-making quality of hexaploid triticale. *Crop Sci.*, 2007, 46(5): 2183-2194 (doi: 10.2135/cropsci2006.03.0135).
- 8. Grebtsova L.N. Novosti nauki Kazakhstana, 2013, 3: 155-159 (in Russ.).
- Ittu Gh., Saulescu N., Ittu M., Mustatea P. Achievements in triticale breeding (× *Triticosecale* Witt.). I.N.C.D.A. *Annals Fundulea*, 2007, 75: 73-82.
- 10. Wos H., Brzezinski W.J., Arseniuk E., Zimny J., Wos J. Triticale of improved bread-making quality *Proc. 18th EUCARPIA General Congress «Modern variety breeding for present and future needs», 9-12 Sep 2008, Valencia-Spain.* Valencia, 2008: 661.
- 11. Diordieva I.P., Parii F.N. Geneticheskie resursy rastenii, 2014, 5: 42-53 (in Ukr.).
- 12. Grabovets A.I., Fomenko M.A. Zernobobovye i krupyanye kul'tury, 2013, 2(6): 41-47 (in Russ.).
- Hills M.J., Hall L.M., Messenger D.F., Graf R.J., Beres B.L., Eudes F. Evaluation of crossability between triticale (× *Triticosecale* Wittmack) and common wheat, durum wheat and rye. *Environ. Biosafety Res.*, 2007, 6: 249-257 (doi: 10.1051/ebr:2007046).
- 14. Dvorak J., Deal K.R., Luo M.C., You F.M., Borstel K.V., Dehghani H. The origin of spelt and free-threshing hexaploid wheat. *The Journal of Heredity*, 2012, 103(3): 426-441 (doi: 10.1093/jhered/esr152).
- Zielinski H., Ceglinska A., Michalska A. Bioactive compounds in spelt bread. *Eur. Food Res. Technolol.*, 2008, 226: 537-544 (doi: 10.1007/s00217-007-0568-1).
- Blatter R.H.E, Jacomet S., Schlumbaum A. Spelt-specific alleles in HMW glutenin genes from modern and historical European spelt (*Triticum spelta* L.). *Theor. Appl. Genet.*, 2002, 104(2-3): 329-337 (doi: 10.1007/s001220100680).
- 17. Guzmán C., Xiao Y., Crossa J., González-Santoyo H., Huerta J., Singh R., Dreisigacker S. Sources of the highly expressed wheat bread making (wbm) gene in CIMMYT spring wheat germplasm and its effect on processing and bread-making quality. *Euphytica*, 2016, 209: 689-692 (doi: 10.1007/s10681-016-1659-5).
- 18. Parii F.N., Diordieva I.P. Sozdanie chetyrekhvidovykh form tritikale. Zemledelie i zashchita rastenii, 2015, 5(102): 35-42.
- 19. Ng P.K.W., Sconlon M.G., Bushuk W.A. Catalog of biochemical fingerprints of registered Canadian wheat cultivars by electrophoresis and high-perpformanse liquid chromatography. Uni-

versity of Manitoba, Winnipeg, 1988

- 20. Shchipak G.V. V sbornike: *Spetsial'naya selektsiya i semenovodstvo polevykh kul'tur* [In: Special selection and seed production of field crops]. Khar'kov, 2010: 70-107 (in Russ.).
- 21. Ermantraut E.R., Gudz' V.P. Materialy Mezhdarodnoi nauchno-prakticheskoi konferentsii «Sovremennye problemy opytnogo dela» [Proc. Int. Conf. «Current problems of experiment design»]. St. Petersburg, 2000: 13-134 (in Russ.).
- 22. Maksimov N.G. Selektsiya i semenovodstvo, 2011, 99: 30-38 (in Russ.).
- 23. Pershina A.A., Trubacheva N.V. Vavilovskii zhurnal genetiki i selektsii, 2016, 20(4): 416-425 (doi: 10.18699/VJ16.082) (in Russ.).
- 24. Ukalska J., Kociuba W. Phenotypical diversity of winter triticale genotypes collected in the Polish gene bank between 1982 and 2008 with regard to major quantitative traits. *Field Crops Research*, 2013, 149: 203-212 (doi: 10.1016/j.fcr.2013.05.010).
- Ayalew H., Kumssa T.T., Butler T.J., Ma X.-F. Triticale improvement for forage and cover crop uses in the southern great plains of the United States. *Front. Plant Sci.*, 2018, 9: 1130 (doi: 10.3389/fpls.2018.01130).
- 26. Barnett R.D., Blount A.R., Pfahler P. L., Bruckner P.L., Wesenberg D.M., Johnson J.W. Environmental stability and heritability estimates for grain yield and test weight in triticale. *J. Appl. Genet.*, 2006, 47: 207-213 (doi: 10.1007/BF03194625).
- 27. Kurkiev K.U. Genetika, 2007, 9: 1269-1272 (in Russ.).