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ORIGINAL ARTICLE

Triticale breeding improvement by the intraspecific and remote hybridization

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The purpose of our research was to expand the genetic diversity and to create the new winter *Triticale* materials with high grain quality by use of intraspecific and remote hybridization, involving them in the breeding of high-yield cultivars. Samples of winter *Triticale* were created by intraspecific and remote hybridization methods using multiple individual selections at Uman National University of Horticulture (Ukraine). We created the collection of winter *Triticale*, which includes more than 300 samples. It consists of unique recombinant forms that differ in morphobiological and economically valuable indicators. The obtained samples were divided according to the plant height into medium-stemmed (over 100 cm), low-stemmed (80–99 cm), short-stemmed (60–79 cm) and dwarf (<60 cm). The sample 68 combined high yield (6.95 t/ha) and grain quality (protein content — 12.8%, gluten — 27.8%, 1000-grain weight — 50.5 g, grain unit — 690 g/l). Sample 83 was characterized by high protein content (14.2%) and gluten (30.2%) of group I, and was up to the standard yield (6.35 t/ha). We created the cultivars of winter *Triticale* 'Navarra' and 'Strateg' that listed in the State Register of Plant Cultivars Suitable for Distribution in Ukraine in 2018.

Keywords: Intraspecific hybridization; Remote hybridization; Spelt wheat; Sand ryegrass; Protein content; Gluten content; productivity

Introduction

Winter *Triticale* (*x Triticosecale* Wittmack) is used as a feed, food and industrial crop. The sown areas of these crops in the world reach 4 million hectares (Estrada-Campuzano et al., 2012; Grebennikova et al., 2016). Priorities for *Triticale* selection are yield enhancement, stability and improvement of grain quality indicators (Shchypak, 2008; Dennett et al., 2016).

Poor quality of *Triticale* grain, in particular low protein content and poor-quality of gluten are a major obstacle to large-scale crop implementation into agricultural production. Therefore, the main objectives of breeding are to improve the quality of grain, which is usually accompanied by a reduction in yield, as these indicators correlate negatively among themselves (Diordiieva et al., 2019a; Zhou et al., 2012; Kang et al., 2012). Nowadays scientists are breeding winter *Triticale* with high yield capacity, resistant to unfavourable environmental conditions, mainly the high grain quality cultivars.

Insufficient plasticity of cultivars and breeding forms of winter *Triticale* related to the limited genetic diversity of parent material requires enlarging the gene pool and increasing breeding efficiency by different methods. It has been established that hybridization of ecologically and geographically distant forms is an effective method of selection, but the success of the work depends heavily on the correct choice of the primary crossing components for creating new forms (Hrebtsova, 2013). Related and distant taxa, wild and semi-wild species, etc. may serve as donors of economically valuable characteristics (Diordiieva et al., 2019b). The extensive formation process provided by remote hybridization can significantly expand the genetic diversity of the parent material and obtain recombinant forms with improved quantitative and qualitative productivity indicators (Hrabovets and Fomenko 2013; Ukalska and Kociuba, 2013). Scientists from all over the world are doing genetic improvement of *Triticale* by remote hybridization. The lines of *Triticale* with multiple chromosomal substituted and high productivity indicators of ears have been created by the crossing of *Triticale* with rye (Hao et al., 2013). There are also examples of successful hybridization of *Triticale* with wheat (*Triticum* L.) (Kang et al., 2016), quackgrass (*Agropyron* repens), sand ryegrass (*Elymus arenarius*), lemon grass (*Cymbopogon*) (Kavanagh et al., 2010), barley (*Hordeum vulgare*) (Zenkteler and Nitzsche, 1984), resulting in the creation of valuable source material.

The purpose of our research was to expand genetic diversity and create new winter *Triticale* materials of high grain quality by intraspecific and remote hybridization and to involve them in the breeding process of creating high-yielding crop cultivars.

Materials and Methods

Winter *Triticale* samples were created by intraspecific and remote hybridization methods using multiple individual selections. In the hybridization system as parent material we included released winter and spring *Triticale* cultivars of domestic ('Rozivska 6', 'Alkid', 'Rarytet', 'Khlibodar Kharkivskyi') and foreign ('Beta', 'Suvenir') breeding; *Triticale* samples of self-selection, selected from hybrid flocks and obtained from gradual crossings of *Triticale* cultivars among themselves and with spelt wheat (*Triticum spelta* L.); samples of octoploid and hexaploid chromosomally substituted (1R/1D) forms of *Triticale* provided by the National Center for Plant Genetic Resources of Ukraine; winter spelt wheat 'Zoria Ukrainy'; sand ryegrass (*Elymus arenarius*). Hybridization was carried out by

manual castration of female flowers and their following controlled pollination with male flowers. Grain harvesting and calculating were carried out in a firm ripe stage.
$F_{2–5}$ hybrid progeny were analyzed for morphobiological and economically valuable features (plant height, ears length and colour, grain weight of the main ear, 1000-grain weight, protein and gluten content of grains, gluten quality, crop yield, etc.). Starting with the fourth generation, the best samples of economically valuable indicators were selected for further testing conducted during 2015–2019. Calculating, phenological observations and defining quality indicators were conducted according to State qualification methodology of plant cultivars expertise on definition of suitability for distribution in Ukraine (2015). Plant height was measured in the field before harvesting. Grouping of *Triticale* samples by plant height was performed according to the classification of V. G. Shchypak (2010). Winter *Triticale* 'Rarytet' was chosen as the standard. In the experiments, a systematic method of plot location with 10 m^2 accounting area was used. Numbered samples were placed in a block design with plant density of 400 thousand units per hectare. The experiment was repeated five times. Biometric parameters were determined on 50 plants, which were taken from each plot in two non-contiguous replications. After calculating and measurements, grain was threshed and the yields were determined. The measurement validity, variation degree and significance of the differences were evaluated according to E.R. Ermantraut and V.P. Gudz (2000).

Results and Discussion

A number of intraspecific and interspecific crossings were conducted during the research. The obtained F_1 hybrids were self-pollinated or crossed with the parental forms. The genetic diversity used in hybridization has provided a wide-ranging process of formation across hybrid generations. Particular attention was paid to the detailed analysis of the material at the beginning of the breeding process, because, as V. S. Kochmarskyi (2012) points out, only recombination variability in $F_{2–4}$ generations ensures that new forms of transgressive plants based on economically valuable features are obtained.
During the individual and family selection, *Triticale* samples were selected among progeny, which were characterized by considerable diversity in morphobiological features (plant height, ear shape and length, plant and ear colour, etc.). The work collection includes forms characterized by early maturity, low stem, high winter and frost resistance and other valuable features. Certain forms exceed the range of variability of parent materials in yield, protein and gluten content of grain, ear productivity, etc. (Table 1). *Triticale* is characterized by high stem, which causes lodging of plants. Therefore, reducing plant height without sacrificing a high level of productivity is an urgent task of the crop breeding. In terms of plant height, the collection includes a wide variety of shapes. The range of variability on this feature was 65–39 cm. In *Triticale* six genes that control plant height have been identified, in particular, dominant and recessive genes of short stem of rye, partially dominant gene introgressed from soft wheat 'Tom Pouce', and three recessive genes of Rht stemness, that are inherited from soft wheat (Kurkyev, 2001). Intraspecific crossings provide different inheritance of characteristics from intermediate to heterosis and dominant dwarfism.
According to the classification of V. G. Shchypak (2010), the created samples were divided into medium-stemmed (over 100 cm), low-stemmed (80–99 cm), short-stemmed (60–79 cm) and dwarf (< 60 cm). The most numerous and productive are low- and medium-stemmed groups. A significant decrease in plant height relative to the standard was observed in five samples, in particular those obtained from crossing of octaploid (samples 33 and 56) with three-species hexaploid *Triticale* and with forms having 1R/1D chromosomal displacement (sample 15), because the initial forms of these progenies according to the height of the plants belonged to the low-stemmed group (80–99 cm).
Significant decrease in height relative to the standard was also observed in samples 61 and 68 (95 and 87 cm, respectively) obtained in crossbreeding combinations, where tall-growth spelt wheat was the parent form. Probably this can be explained by the presence of dominant rye genes of short-stem trait in the initial *Triticale* form, as it was found that these genes were epistatic in relation to others controlling the height of plants.
Creating *Triticale* samples of well-grained ear is an important task for plant breeding, as grain productivity is closely related to grain weight per ear. In addition, the possibility of increasing grain number per ear is possible by increasing its length, because in some cases the long ear forms 250–300 grains (Diordiieva et al., 2019). For that purpose, sand ryegrass (*Elymus arenarius*) characterized by long ear and high grain productivity was involved in the hybridization system. The progenies obtained with the participation of this species (sample 8) have formed an elongated inflorescence by 8.2 cm and increase in grain number per ear by 14 grains. However, this sample showed a decrease in grain size, which negatively affected the grain weight per ear (1.75 g) and grain yield (5.78 t/ha). The average level of variation was recorded by the grain weight per ear (V = 12.1%). Samples 15, 24, 35, 58, 63 and 68 significantly exceeded the standard for this indicator. By the grain number per ear samples 8, 63 and 83 significantly exceeded Rarytet variety. This figure among the other samples tested, except for 56, was at the standard level. Proved increase in crop yield relative to the standard was recorded in samples 35 (6.81 t/ha), 58 (7.25 t/ha), 61 (6.92 t/ha), 63 (7.01 t/ha) and 68 (6.95 t/ha).

Table 1. Economically valuable characteristics of winter *Triticale* samples, 2015–2019.

Sample	Origin	Plant height, cm	Ear length, cm	Grain weight per ear, g	Number of grains per ear, pcs.	Yield capacity, t/ha
Rarytet (st)	Plant Production Institute named after V.Ya. Yuriev of NAAS	110	13.2	2.03	50	6.48
8	*Triticosecale* Wittmack[1] × *Elymus arenarius*	115	22.4	1.75	64	5.78
15	*Triticosecale* Wittmack[1] × *Triticosecale* Wittmack (1R/1D)[4]	105	12.7	2.12	52	6.65
28	*Triticosecale* Wittmack[1] × *Triticum spelta* L.	112	14.2	2.08	48	5.87
33	*Triticosecale* Wittmack (2n=56. UA0602463[2]) × *Triticosecale* Wittmack[1]	98	12.8	2.01	50	6.41
35	*Triticosecale* Wittmack ('Beta') × *Triticum spelta* L.[3]	115	13.2	2.35	53	6.81
56	*Triticosecale* Wittmack (2n=56.	95	13.5	2.05	45	6.58

	UA0602463[2]) × *Triticosecale* Wittmack[1]					
58	*Triticosecale* Wittmack ('Alkid') × *Triticosecale* Wittmack ('Suvenir')	107	12.5	2.38	52	7.25
61	*Triticosecale* Wittmack[1] × *Triticum spelta* L. [3]	95	12.0	2.10	47	6.92
63	*Triticosecale* Wittmack* × *Triticosecale* Wittmack (UA0601654)[2]	118	11.5	2.22	54	7.01
68	*Triticosecale* Wittmack[1] × *Triticum spelta* L. [3]	87	12.8	2.15	52	6.95
455	*Triticosecale* Wittmack[1] × *Triticum spelta* L. [3]	108	12.8	1.98	54	6.35
491	*Triticosecale* Wittmack ('Khlibodar Kharkivskyi') × *Triticum spelta* L. [3]	110	13.4	2.25	51	6.53
	$LSD_{0.95}$	4	0.5	0.07	3	0.22
	V,%	22	15.8	12.1	8	9.22
	Sx,%	3.7	3.8	3.5	4.0	3.68

Here and then: [1] – *Triticale* samples of self-breeding selected from hybrid populations obtained from gradual crossing of a number of *Triticale* cultivars with each other and with spelt wheat; [2] – sample number according to the Catalog of the National Center for Plant Genetic Resources of Ukraine; [3] – in the hybridization system winter wheat 'Zoria Ukrainy' was used; [4] – hexaploid form of *Triticale* with 1R/1D type chromosome displacement

The main purpose of the research was the genetic improvement of *Triticale*, the increase of protein and gluten content in the grain, which would improve its baking and technological properties. A number of scientists have shown that *Triticale* grain contains 10–12% of protein and 20–25% of gluten; this is considerably less than similar indicators of soft winter wheat (Grebennikova et al., 2016; Shchypak, 2008; Ukalska and Kociuba 2013; Lukaszewski, 2007; Wos et al., 2008). However, scientists (Ayalew et al., 2016) also note the high potential for simultaneous improvement of crops on productivity indicators and grain quality.

As a result, eight samples of *Triticale* were obtained, which in the grain protein and gluten content significantly exceeded the standard; two samples were at the level of the standard, one sample significantly inferior to it. Samples obtained by hybridization of three-species *Triticale* with spelt wheat (28, 68, 83) and hexaploid forms with octaploid (33, 56) were characterized by the highest protein and gluten content (Table 2).

Spelt wheat (genomic formula AABBDD) and octaploid *Triticale* (genomic formula AABBDDRR) contain a complete set of chromosomes of the D-genome, which are known to significantly affect the quality and baking properties of the grain. Their hybridization with three-species hexaploid *Triticale* (genomic formula AABBRR) provides independent recombination of genetic material, resulting in new transgressive forms of progenies with improved quality indicators.

Gluten quality is important in baking. Physical indicators of quality, such as colour, elongation, elasticity, and deformation index significantly affect the dough formation and bread yield. According to DSTU 3768: 2010 (2010), gluten of the first quality group must be of light grey or grey colour, elastic, with an elongation of 10–20 cm and have gluten deformation index within 45–85 units of gluten deformeter. The quality group may be reduced if the deformation index exceeds the limits allowed for group I, as gluten deformation index is the most important indicator of gluten quality.

Table 2. Sample characteristics of grain quality of winter *Triticale*, 2015–2019.

Sample	Origin	1000-grain weight, g	Grain-unit, g/l	Protein content, %	Gluten	
					%	Quality group
Rarytet (st)	Plant Production Institute named after V.Ya. Yuriev of NAAS	48.5	680	11.8	24.2	I
8	*Triticosecale* Wittmack[1] × *Elymus arenarius*	43.1	620	11.4	24.0	II
15	*Triticosecale* Wittmack[1] × *Triticosecale* Wittmack (*1R/1D*) [4]	48.8	650	12.2	25.5	I
28	*Triticosecale* Wittmack[1] × *Triticum spelta* L.	49.1	670	13.0	26.6	II
33	*Triticosecale* Wittmack (2n = 56, UA0602463[2]) × *Triticosecale* Wittmack[1]	49.5	660	12.8	26.4	II
35	*Triticosecale* Wittmack ('Beta') × *Triticum spelta* L. [3]	48.5	690	12.4	23.8	II
56	*Triticosecale* Wittmack (2n = 56, UA0602463[2]) × *Triticosecale* Wittmack[1]	49.2	660	12.6	26.3	II
58	*Triticosecale* Wittmack ('Alkid') × *Triticosecale* Wittmack ('Suvenir')	50.3	700	11.2	22.8	II
61	*Triticosecale* Wittmack[1] × *Triticum spelta* L. [3]	49.4	660	12.4	26.0	II
63	*Triticosecale* Wittmack[1] ×	49.8	680	12.2	25.4	I

	Triticosecale Wittmack (UA0601654)[2]					
68	*Triticosecale* Wittmack[1] × *Triticum spelta* L. [3]	50.5	690	12.8	27.8	I
455	*Triticosecale* Wittmack[1] × *Triticum spelta* L. [3]	48.0	700	14.2	30.2	I
491	*Triticosecale* Wittmack[1] × *Triticosecale* Wittmack ('Khlibodar Kharkivskyi')	48.2	680	12.5	26.4	I
	$LSD_{0.95}$	1.7	25	0.4	0.9	—
	V,%	5.6	15.1	10.1	10.8	—
	Sx,%	3.5	4.0	3.6	3.7	—

We revealed that five samples had gluten of the first group. Sample 83 is worth noting, which was characterized by the highest protein content (14.2%) and gluten (30.2%) of the 1st group and was not inferior to the yield of the standard (6.35 t/ha).
By 1000-grain weight, 10 tested samples did not differ significantly from the standard, except for the sample 8 (43.1 g) obtained with the presence of sand ryegrass. The presence of sand ryegrass genetic material in the genome, although it leads to the improvement of grain weight per ear, but still has a negative effect on the size and fullness of the grain. Samples 58 and 83 (700 g/l) were distinguished by the highest grain grain-unit.
The established collection samples are constantly being tested. The search for the new valuable donor-forms is ongoing. As a result of the conducted research, Navarra and Strateg cultivars of winter *Triticale* were created and listed in the State Register of Plant Cultivars suitable for distribution in Ukraine from 2018. Breeding Sample 68 was selected, which, after comprehensive study and reproduction, is planned to be submitted to the State scientific and technical expertise.
'Strateg' (sample 455) was created by the gradual hybridization of three-species hexaploid *Triticale* ('Rozovska' 6 and 'Alkid' cultivars) among themselves, followed by the crossing of progenies with spelt wheat ('Zoria Ukrainy'). Subvariety: *Lutescens*. Winter-type growth. As to the ploidy value it refers to hexaploids (2n = 6x = 42). The variety has partial *1RS/1AL* wheat and rye chromosomal replacement. It belongs to the middle-early plant group with a vegetation period of 275–280 days. It is characterized by even plant stand and even maturing. The bush is half-upright, the plants are high with a waxy coating. The ear is semi-clavate, long (13.8 cm), of low-density (16.0 spikelet/10 cm of the ear), white, beardless in the phase of full ripeness. The kernel is egg-shaped, large, light brown in color. The variety is characterized by high resistance (8.0–9.0 points) against farinaceous food, Fusarium head blight, root rot, brown rust, lodging and shedding. During the period of the State scientific and technical expertise (2015–2018), the yield was 5.03 t/ha in the Polissia zone, and 5.13 t/ha in the Forest-steppe zone of Ukraine.
'Navarra' variety (sample 491) was created by the hybridization of winter *Triticale* 'Rozivska 6' with spelt wheat, by the crossing of progenies with pollen of spring *Triticale* 'Khlibodar Kharkivskyi'. Subvariety: *Erythrospermum.* As to the ploidy value it refers to hexaploids (2n = 6x = 42). It belongs to the middle-early plant group with a vegetation period of 275–280 days. It is characterized by even plant stand and even maturing. The bush is half-upright, the plants are high with a waxy coating. The ear is cylindrical, medium length (11.4 cm) and low-density (16.0 spikelet/10 cm of the ear), white, awned, beardless in the phase of full ripeness. The kernel is egg-shaped, medium-sized, light brown in colour. Winter-type growth. The variety is characterized by high resistance (8.0–9.0 points) against farinaceous food, Fusarium head blight, root rot, brown rust, lodging and shedding. During the period of the State scientific and technical expertise (2015–2018), the yield was 5.46 t/ha in the Polissia zone, and 5.26 t ha^{-1} in the Forest-steppe zone of Ukraine. Sample 68 was created by the hybridization of three-species winter hexaploid *Triticale* of self-breeding with spelt wheat. It belongs to the low-stemmed form (87 cm). Maturity group: middle-early (290–295 days). The ear is awned, medium length, high-density, white. It is characterized by high yield (6.95 t/ha) and grain quality, in particular, protein content of 12.8%, gluten of the 1st quality group of 27.8%, 1000-grain weight of 50.5 g, grain unit of 690 g/l.

Conclusion

We created the collection of winter *Triticale* samples by the methods of intraspecific and remote hybridization, comprising more than 300 specimens. It consists of unique recombinant forms that differ in morphobiological and economically valuable indicators. We selected sample 68, which combines high yield (6.95 t/ha) and grain quality (protein content —12.8%, gluten — 27.8%, 1000-grain weight —50.5 g, grain unit — 690 g/l). We also created the cultivars of winter *Triticale* 'Navarra' and 'Strateg' that were listed in the State Register of Plant Cultivars Suitable for Distribution in Ukraine in 2018.

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