

## NEW GENOTYPES AND TECHNOLOGICAL INDICATORS OF WINTER TRITICALE

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Received 19.02.2016

The aim of the research was to conduct basic screening of new lines and cultivars of winter hexaploid triticale by the technological and molecular genetics indicators. Molecular and genetic research conducted by polymerase chain reaction allelic variants of gene loci *Wx-A1*, *Wx-B1*, and quality parameters of grain, flour and bread — on technological markers. The new cultivars and lines of winter hexaploid triticale of Nosivka Breeding and Research Station of Remeslo Myronivka Institute of Wheat by technological indicators of grain, flour and bread quality were studied. According to representative criteria's the most promising genotypes, which are the main products in terms Forest-Steppe ecotypes' and a high-quality raw materials for bakeries and bioethanol were identified. Molecular and genetic identifications of allelic variants of genes loci *Wx-A1*, *Wx-B1* triticale, which in the early stages of ontogenesis to predict targeted uses genotypes were conducted. The first among a series of triticale cultivars and lines Forest-Steppe ecotypes and biotypes with nonfunctional "b" gene allele *WxA1*, which defines a high content of amylopectin of starch, an important release for more ethanol was identified. It was found that technological characteristics of grain, flour and bread of new cultivars and lines of winter triticale meet the modern requirements production dietetic food and bioenergy products is important and relevant in the context of food security of Ukraine.

**Key words:** winter triticale, molecular and genetic markers.

Triticale is a promising culture for expanding the resource base of baking industry and bioethanol production. This is a separate plant genus *x Triticosecale* created by artificial selection (from *Triticum* — the wheat genus, and *Secale* — the rye genus) [1–3]. This artificial plant is of interest due to its nutritional value which largely surpasses in many aspects those of wheat and rye. Nutritional value of triticale grain is caused by high content of protein, essential amino acids, minerals, vitamins B, PP, E, and carotenoids. It should also be noted that the triticale culture is not very discriminative of growing conditions and is the most capable among other crops in the area of weak implementation of biological potentials. Currently, there is a number of winter and spring triticale sorts with high productivity, grain quality, and stable expression of economic characters [4–6]. Not all of the sorts present the

valued economic characteristics that meet the requirements of baking and alcohol-distilling use [7, 8]. Thus expansion of gene pool of triticale with such properties is important for national nutritional safety.

The aim of the work was to screen new lines and sorts of hexaploid winter triticale by technologic features and molecular genetic markers.

### Materials and Methods

In 2008–2015, we bred new lines and sorts of winter triticale (Pshenychny, Slavetny, Chaian, Д-5\_2010, ПС\_2-12, ПС\_1-12) and studied their genotypic and phenotypic characters in the conditions of Polissia and Forest-Steppe ecotopes (Nosivka selection and research station of Remeslo Myronivka Institute of Wheat of NAAS of Ukraine).

Evaluation of triticale grain and flour by the cereal, protein-proteinase, and carbohydrate-amylase complexes, and of the triticale bread was done in collaboration with Yuriev Plant Production Institute of NAAS of Ukraine in the grain quality laboratory. Extraction of total DNA from triticale grain was conducted according to [9–11], with certain modifications. Gene polymorphism analysis was performed using uniplex and multiplex PCR with specific primers by pre-designed and optimized programs [16]. Reaction solutions were: specific primers, 2 mcl of buffer for PCR *10xDreamTaq™ Green Buffer* (*Thermo Scientific*), 0,2 mM of skin deoxyribonucleotide-3-phosphate (*Scientific*), 0,5 unites of polymerase *DreamTaq™ DNA Polymerase* (*Thermo Scientific*), 30 ng of total plant DNA, de-ionized water *Milli-Q* till the end volume of 20 mcl. Selecting valuable genotypes with zero alleles of *Wx*-genes was done amplifying DNA with primers according to Vanzetti et al., (2009) and Saito et al., (2004) [10, 11], allowing for detection of *Wx-A1b*, *Wx-B1b* alleles. The characteristics of *Wx* genes and alleles of triticale are given in Table 1.

Mathematical and statistical analysis was conducted according to Dospekhov [12] using *Excel 2007* and *Statistica 6.0.* software.

### Results and Discussion

As determined by long-term studies, some of the triticale genotypes such as АД 256 (national standard), Pshenychne, Slavetne, Chaian, Д\_5\_2010, ПС\_2-12, ПС\_1-12 can in Polissia and Forest-Steppe conditions produce high stable harvests of quality grain if grown using scientifically based technologies. It was shown that the abovementioned genotypes have high bakery performance, for example, the grain nature is 690–720 g/l and 35–44% vitrescence (flour quality indicators) (Table 2).

Analysis of technological indicators of grain revealed that the new genotypes lead to the development of grain consisting of 12.1–15.5% protein, 22.6–29.3% gluten (Table 3). In particular, the flour of all studied new genotypes of winter triticale is first-class due to > 18% gluten content. The minimum indicator values of protein complex are in the Д\_5-2010 line.

Quality baking industry depends on the dough elasticity. It is determined that the aforementioned genotypes (namely, АД 256, Slavetne, Д\_5-2010, and Chaian), are characterized by dough elasticity of 52–65 mm, which eventually positively reflects on the total baking assessment of 6.5–8.6 points (Table 3, Fig. 1–4).

Experimentally it was shown that the content of “raw” protein for all studied grain genotypes varies insignificantly (except for Slavetne sort where this indicator is only 12.1% although this is within the error relative to АД 256 control).

Among the important characteristics of quality of triticale grain is the quality of gluten as a combination of physical properties: elasticity, flexibility and extensibility. The resistance to compression deformation of gluten of these triticale varieties and lines (85–106 cond. u.) is much higher than that of the common wheat of Yuivivata sort (60–72 cond. u.). Although the grain of all studied genotypes by the resistance to compression deformation of gluten belongs to the II quality group and is characterized as “satisfactorily weak”, it can be improved with oxidizing supplements that change gluten.

It should be noted that weak gluten has high extensibility, minimum elasticity, and flows quickly [3, 5, 7].

Triticale grain quality depends not only on the content and quality of gluten, but also on the grain carbohydrate-amylase complex (Table 4). Studying the sort peculiarities of starch content in grain and the carbohydrate-

Table 1. Localization of *Wx* genes and alleles in the genome of hexaploid triticale [9–11]

Gene	Allele	Amplicon size, n. p.	Chromosome
<i>Wx-A1</i>	<i>Wx-A1a</i>	495	7AS
	<i>Wx-A1b</i>	652	
	<i>Wx-A1e</i>	176	
<i>Wx-B1</i>	<i>Wx-B1a</i>	778	4AL
	<i>Wx-B1e</i>	934	
	<i>Wx-B1b</i>	668	

Table 2. Performance cereal properties of grain of winter triticale and common wheat

Sort or line	Grain performance indicators			
	moisture content, %	weight of 1000 grains, g	bulk density, g/l	vitrescence, %
ПЦ_1-12	14	58 ± 1.8* "	715 ± 4.1"	35 ± 0.6"
ПЦ_2-12	14	64 ± 2.2* "	720 ± 2.4"	40 ± 0.7* "
Slavetne	14	52 ± 3.9	716 ± 3.0"	13 ± 0.8* "
Chaian	14	49 ± 2.0	695 ± 5.2* "	44 ± 1.0* "
Д-5_2010	14	48 ± 2.3	690 ± 2.7* "	29 ± 0.8* "
Pshenychne	14	55 ± 2.5* "	712 ± 3.3* "	38 ± 0.9* "
АД 256 (control)	14	48 ± 3.9	718 ± 2.0	35 ± 1.1
Yuvivata 60 (wheat)	14	50 ± 2.4	786 ± 1.6	50 ± 0.7

Note: hereafter \* —  $P < 0.05$  versus control (АД 256); " —  $P < 0.05$  versus wheat.

Table 3. Special (technological) indicators of grain quality of winter triticale and common wheat by protein-proteinase complex

Sort, line	"Raw" protein content, %	Raw gluten content, %	Dough extensibility, mm	Dough elasticity, mm	Resistance to compression deformation ( $H_{def}^{MDG}$ , c.u.)	Flour strength, u. a.
ПЦ_1-12	14.9 ± 0.8*	27.7 ± 0.5*"	53 ± 2.2* "	92 ± 3.2* "	90 ± 4.5* "	127 ± 3.0* "
ПЦ_2-12	13.7 ± 0.5	29.3 ± 1.0*	41 ± 2.7* "	78 ± 2.8* "	88 ± 3.0* "	119 ± 2.1* "
Slavetne	12.1 ± 0.6"	24.0 ± 0.6"	34 ± 3.4* "	65 ± 2.0* "	95 ± 3.6* "	72 ± 2.6"
Chaian	14.5 ± 0.5*	26.4 ± 0.4*"	25 ± 1.1"	52 ± 2.1*	95 ± 2.8* "	94 ± 3.4* "
Д-5_2010	12.7 ± 0.5"	22.6 ± 0.7"	27 ± 2.7"	55 ± 2.5*	97 ± 2.1"	70 ± 1.8* "
Pshenychne	15.5 ± 0.7*"	28.6 ± 0.5*	48 ± 2.8* "	90 ± 2.5* "	85 ± 2.7* "	121 ± 2.6* "
АД 256 (st)	12.8 ± 0.4	23.8 ± 0.4	28 ± 2.4	59 ± 3.3	101 ± 2.5	75 ± 2.2
Yuvivata 60	14.2 ± 0.5	29.6 ± 0.5	82 ± 2.5	53 ± 2.7	102 ± 2.0	272 ± 3.4

Table 4. Winter triticale and common wheat grain characteristics by the carbohydrate-amylase complex

Sort, line of triticale	Starch content, %	Amilose content in starch, %	Fermentability of starch, %	Autolytic activity, %, per dry matter	Temperature of maximum viscosity of starch gel, $T_{maxvic}$ , °C	Falling number, c
ПЦ_1-12	68.5 ± 0.6*	22.2 ± 0.7	28.5 ± 0.5	35.5 ± 0.9	83.5 ± 0.7*	178 ± 14.4
ПЦ_2-12	64.7 ± 1.1"	22.6 ± 0.6	29.2 ± 0.8	33.2 ± 1.4	83.0 ± 0.6*	181 ± 13.0
Slavetne	65.8 ± 0.8"	21.4 ± 0.8	27.2 ± 0.8"	35.0 ± 1.2	84.9 ± 0.4*	176 ± 8.2
Chaian	64.0 ± 0.5"	26.2 ± 0.9* "	35.5 ± 0.7* "	41.8 ± 1.0* "	80.4 ± 0.8	172 ± 9.6
Д-5_2010	65.6 ± 0.9"	18.8 ± 0.6* "	23.2 ± 0.7* "	45.6 ± 0.8* "	86.2 ± 0.3*	199 ± 11.7
Pshenychne	67.6 ± 0.5*	23.4 ± 1.3	30.5 ± 1.0	34.7 ± 1.5	80.7 ± 0.4	170 ± 9.6
АД 256 (st)	65.2 ± 0.8	21.9 ± 0.7	28.0 ± 0.8	36.6 ± 1.4	84.1 ± 0.3	181 ± 8.0
Yuvivata 60	67.8 ± 0.6	—	—	—	—	—



Fig. 1. Ear, grain and bread of Pshenychny line of winter triticale



Fig. 2. Ear and bread of Chaian line of winter triticale



Fig. 3. Ear, grain and bread of Slavetne line of winter triticale



Fig. 4. Ear and bread of Д-5-2010 line of winter triticale

amylase complex allowed to determine that the starch content in the grain of aforementioned sorts and lines of triticale is quite high and ranges 64.0–68.5%. The starch is the main grain component, which is why its content, state and properties always impact the rheological properties of dough and therefore the quality of the final products. It should be noted that triticale grain contains a significant total amount of sugars and has a lower temperature of starch gelatinization, and highly active  $\alpha$ -amylase [13].

Lately the Ukrainian baking industry practices the method of estimation of falling number index by viscosity, as is widely

referred to in other countries [5, 14] as як “Hagberg falling number” (*HFN*). This index reflects the activity of special enzymes, mostly  $\alpha$ -amylases that that break down starch molecules into sugars with intense release of  $\text{CO}_2$ , influencing the porosity and bread structure. The preferable  $\alpha$ -amylase level is low [3, 7] because otherwise there is active re-synthesis of starch and the dough becomes viscose and sticky.

High falling number is an important indicator of baking quality of triticale grain, caused by low autolytic activity of  $\alpha$ -amylase enzymes that convert starch amylose into glucose and maltose [5, 7] resulting in high protein content.

Our experiments reveal that the least autolytic activity of the studied sorts and lines of triticale grain is in lines ПС\_1-12 and Pshenychne (33.2 and 34.7% respectively) and the maximum is in Д-5\_2010 (Table 4) which is characterized by the highest activity of amylases and  $\alpha$ -amylase in particular and therefore is the least suitable for the production of bakery products.

High amylase content in the grain of studied triticale genotypes is confirmed by identification, in all sorts and lines, of wild type alleles coding isoforms of *GBSS 1* enzyme (*granulebound starch synthase = ADP glucose starch glycosyl transferase*, EC2.4.1.21 = *GBSS 1*). The relevant genes are located in 4AL chromosome shoulders [12–14, 19]. In particular, the alleles “a” (amplification size 495 n. p.) and “e” (176 n. p.) in the *Wx-A1* gene locus, and alleles “a” (778 n. p.) and “e” (934 n. p.) of *Wx-B1* gene locus (Fig. 5, 6) were found.

Molecular genetic screening for winter triticale grain by alleles of *Wx*-genes confirmed the physical and chemical composition of starch. Particularly, in the Д-5\_2010 the not-functioning null allele “b” of *Wx-A1* gene was found, 652 n. p. in size, with the 5'-CGGCGTCGGG TCCATAGATC-3' sequence (Fig. 6), which does not influence the expression of synthesis of granulebound starch synthase.

The 668 n.p. amplicon — product of *Wx-B1b* gene — was not found in any of studied samples of winter triticale.

Amylopectin, which is part of the starch granules, is hydrolyzed by amylase enzymes better than the amylose [15–19]. Starch granules of amylopectin are physically unstable against mechanical stress during grain grinding, so they are easily broken and thus the total surface of the starch granules that are attacked by amilases increases significantly [13, 20]. This causes the acceleration of starch hydrolysis by amylase, which is important in shaping the raw material for alcohol- distillate and bakery production [2, 16]. Temperature of maximum viscosity of starch gel shows the ratio of amylose and amylopectin polysaccharides in starch granules. The results of our studies on reciprocal interdependence of temperature values of maximum viscosity of starch gel and amylose content and fermentability are consistent with data of some other authors [17, 18]. By temperature values of maximum viscosity of starch gel ( $T_{max}$ ), the grains of new winter triticale genotypes are variable, as confirmed by different amylose contents in studied samples (Table 5). The only exception is the triticale line Д-5\_2010 with  $T_{max}$  higher approximately 7.2% than in others, and with the highest amilopectin level in starch

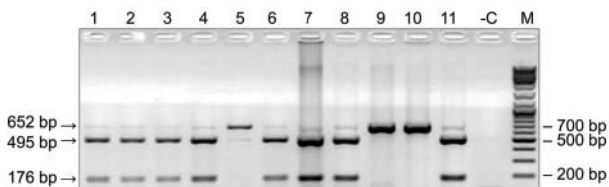


Fig. 5. Electrophoregram of *Wx-A1* gene amplification products:

- 1 — ПС\_1-12;
- 2 — ПС\_2-12;
- 3 — Slavetne;
- 4 — Chaian;
- 5 — Д-5\_2010;
- 6 — Pshenychne;
- 7 — АД 256;
- 8 — control sample of triticale with wild type allele (*Wx-A1a*);
- 9 — control sample of triticale with null allele of *Wx-A1b* gene;
- 10 — control sample of wheat with null allele of *Wx-A1b* gene;
- 11 — control sample of wheat with wild type allele (*Wx-A1a*), the Gardemarin sort;
- C — negative control (without DNA);
- M — molecular weight marker GeneRuler™ DNA Ladder Mix

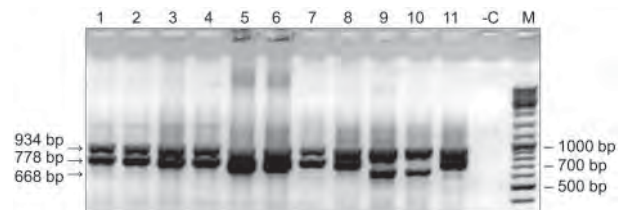


Fig. 6. Electrophoregram of *Wx-B1* gene amplification products:

- 1 — ПС\_1-12;
- 2 — ПС\_2-12;
- 3 — Slavetne;
- 4 — Chaian;
- 5 — Д-5\_2010;
- 6 — Pshenychne;
- 7 — АД 256;
- 8 — control sample of triticale with wild type allele (*Wx-A1a*);
- 9 — control sample of triticale with null allele of *Wx-B1b* gene;
- 10 — control sample of wheat with null allele of *Wx-B1b* gene;
- 11 — control sample of wheat with wild type allele (*Wx-A1a*), the Gardemarin sort;
- C — negative control (without DNA);
- M — molecular weight marker GeneRuler™ DNA Ladder Mix

Table 5. Average indicators of quality of winter triticale and winter soft wheat bread

Sort, line	Moisture content, %	Acidity, pH	Porosity, %	Volumetric bread output of 100 g of dough, cm <sup>3</sup>	Total baking rating, points	Specific volume, cm <sup>3</sup> /100 g
ПЦ_1-12	54.0 ± 0.2* "	5.1 ± 0.1 "	41.1 ± 1.3 "	490 ± 5.2* "	9.0 ± 0.3*	1.37 ± 0.1 "
ПЦ_2-12	53.7 ± 0.1* "	5.0 ± 0.2 "	39.6 ± 1.7 "	470 ± 4.4* "	8.5 ± 0.4	1.35 ± 0.1 "
Slavetne	55.6 ± 0.4 "	5.6 ± 0.1 "	40.5 ± 1.4 "	460 ± 3.8* "	8.6 ± 0.6	1.34 ± 0.2 "
Chaian	52.2 ± 0.2* "	4.8 ± 0.2 "	39.3 ± 2.6 "	430 ± 3.0* "	8.2 ± 0.6	1.31 ± 0.1 "
Д-5_2010	56.8 ± 0.3* "	6.1 ± 0.3* "	25.8 ± 1.1* "	390 ± 6.6* "	6.5 ± 0.3* "	1.20 ± 0.1 "
Pshenychne	53.0 ± 0.3* "	5.2 ± 0.1 "	40.4 ± 2.0 "	480 ± 6.1* "	9.0 ± 0.5	1.34 ± 0.1 "
АД 256	55.1 ± 0.2	5.3 ± 0.4	40.2 ± 1.6	440 ± 5.3	8.0 ± 0.4	1.33 ± 0.2
Yuvivata 60	50.2 ± 0.3	3.5 ± 0.3	64.8 ± 2.4	590 ± 6.5	8.7 ± 0.6	3.24 ± 0.2

granules (81.2%), which is indicative of valuable raw material for bioethanol industry.

Analysis of quality indicators of products made it possible to identify the best bakery triticale genotypes: ПЦ\_1-12, ПЦ\_1-12, Chaian, Pshenychne, АД 256. Bread, made from grain of triticale line Д-5\_2010 was of worse quality. This is due to low content of raw gluten and increased amylase activity. In addition, low gluten content in grain triticale lines indicated poor porosity makes bread. In addition, low gluten content in grain of that triticale line causes poor bread porosity. The biggest porosity of 39.3-41.1% characterized the bread made of triticale grain of ПЦ\_1-12, Slavetne, Pshenychne lines. The bread with highest specific volume (1.37 cm<sup>3</sup>/g) was made from triticale flour of ПЦ\_1-12 line, characterizing it as the most promising for baking. Pshenychne and ПЦ\_2-12 triticale lines, characterized by high levels of protein-proteinase and carbohydrate-amylase complex, perform well in bakery products and are also noteworthy.

Hence, here molecular and genetic markers and technological indicators of grain, flour and bread of new sorts and lines of hexaploid winter triticale were studied at

Nosivka Breeding and Research Station of Remeslo Myronivka Institute of Wheat of the National Agrarian Academy of Sciences of Ukraine. Of the triticale genotypes grown in Polissia and forest-steppe ecotope conditions, the most promising genotypes were chosen by relevant criteria in order to determine producers of quality raw material for baking and bioethanol industry. Identification of allelic variants of genes Wx-A1, Wx-B1 allows to determine the targeting use of genotypes at the early stages of ontogeny. For the first time, among a number of triticale varieties and lines grown in Polissia and Forest-Steppe ecotopes, biotypes with nonfunctional (*A1b*) allele of *Wx* gene which defines the high content of amylopectin in starch of the grain was identified, which is important for increasing the output of bioethanol.

It is found that high-performance protein-proteinase and carbohydrate-amylase complexes, and qualitative assessment of bakery products as well as of new varieties of winter triticale lines meet modern requirements for effective processing and production of important dietary food products, which is important and a priority in the context of food security of Ukraine.

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## НОВІ ГЕНОТИПИ ТА ТЕХНОЛОГІЧНІ ПОКАЗНИКИ ТРИТИКАЛЕ ОЗИМОГО

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Метою роботи було вивчення нових ліній та сортів гексаплоїдного тритикале озимого за технологічними показниками та молекулярно-генетичними маркерами. Молекулярно-генетичні дослідження проведено за допомогою полімеразної ланцюгової реакції алельних варіантів локусів генів *Wx-A1*, *Wx-B1*, а якісні параметри зерна, борошна й хліба — за технологічними показниками. Вивчено нові сорти та лінії гексаплоїдного тритикале озимого Носівської селекційно-дослідної станції Миронівського інституту пшениці ім. В. М. Ремесла НААН України за технологічними показниками якості зерна, борошна й хліба. Ідентифіковано найбільш перспективні генотипи тритикале, основна продукція яких в умовах полісько-лісостепового екотопу є якісною сировиною для виготовлення хлібопродуктів і біоетанолу. Проведено молекулярно-генетичну ідентифікацію алельних варіантів локусів генів *Wx-A1*, *Wx-B1* тритикале, яка дає змогу на ранніх етапах онтогенезу спрогнозувати цільове використання генотипів. Уперше серед низки сортів і ліній тритикале поліського і лісостепового екотипів ідентифіковано біотипи з нефункціональним алелем «b» гена *WxA1*, який визначає високий вміст амілопектину в крохмалі, що є важливим для більшого виходу біоетанолу. З'ясовано, що технологічні показники зерна, борошна та хліба нових сортів і ліній тритикале озимого відповідають сучасним вимогам виробництва дієтично-харчових і біоенергетичних продуктів, що є актуальним та пріоритетним у контексті продовольчої безпеки України.

**Ключові слова:** тритикале озиме, молекулярно-генетичні маркери.

## НОВЫЕ ГЕНОТИПЫ И ТЕХНОЛОГИЧЕСКИЕ ПОКАЗАТЕЛИ ТРИТИКАЛЕ ОЗИМОГО

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Целью работы было изучить новые линии и сорта гексаплоидного тритикале озимого по технологическим показателям и молекулярно-генетическим маркерам. Молекулярно-генетические исследования проведены с помощью полимеразной цепной реакции аллельных вариантов локусов генов *Wx-A1*, *Wx-B1*, а качественные параметры зерна, муки и хлеба — по технологическим показателям. Изучены новые сорта и линии гексаплоидного тритикале озимого Носовской селекционно-опытной станции Мироновского института пшеницы имени В. М. Ремесло НААН Украины по технологическим показателям качества зерна, муки и хлеба. Идентифицированы наиболее перспективные генотипы, основная продукция которых в условиях полесско-лесостепного экотопа является качественным сырьем для изготовления хлебопродуктов и биоэтанола. Проведена молекулярно-генетическая идентификация аллельных вариантов локусов генов *Wx-A1*, *Wx-B1* тритикале, что позволяет на ранних этапах онтогенеза спрогнозировать целевое использование генотипов. Впервые среди ряда сортов и линий тритикале полесского и лесостепного экотипов идентифицированы биотипы с нефункциональным аллелем «b» гена *WxA1*, который определяет высокое содержание амилопектина в крахмале, что важно для более высокого выхода биоэтанола. Установлено, что технологические показатели зерна, муки и хлеба новых сортов и линий тритикале озимого соответствуют современным требованиям производства диетически-пищевых и биоэнергетических продуктов, что является актуальным и приоритетным в контексте продовольственной безопасности Украины.

**Ключевые слова:** тритикале озимое, молекулярно-генетические маркеры.