ISSN 1068-3674, Russian Agricultural Sciences, 2009, Vol. 35, No. 1, pp. 1–4. © Allerton Press, Inc., 2009.
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Dynamics of the Composition of Gliadin Biotypes during Creation of the Spring Triticale Cultivar Zolotoi Grebeshok

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Received April 1, 2008

Abstract—The dynamics of the composition of gliadin biotypes of a Mexican population of triticale Merino/Jlo/Zebra32 during selection for a set of economically valuable traits under northwestern Russia conditions is studied. The characteristics of the structure of the cultivar Zolotoi Grebeshok created on this basis are registered in the form of a "protein passport" that gives additional information for legal protection of the cultivar and allows checking its purity and authenticity at all stages of seed production.

DOI: 10.3103/S1068367409010017

In the northwestern region of Russia, winter cereals very often can't realize advantages with respect to yield owing to a complex set of unfavorable overwintering conditions. At the same time, spring triticale can be grown here as an exceptionally promising feed grain crop. The selection of high-yielding forms well adapted to local conditions from heterogeneous accessions of spring triticale of the VIR (Vavilov All-Russian Research Institute of Plant Industry) world collection is effective for accelerated creation of the required cultivars. In this case, it is necessary to analyze the structure of the initial population and it dynamics during selection for a set of valuable traits. Along with field assessments, such a check is possible by means of molecular markers, including protein ones (electrophoretic spectra of gliadin) [1–3].

As a result of research at VIR's Pushkino branch (Leningrad oblast, 1999–2001) of more than 200 spring triticale accessions of various ecogeographic provenance, a Mexican accession Merino/Jlo/Zebra32 (k-3503) was revealed which combines an acceptable length of the growth period (90–95 days) with high productivity, excellently filled grain, and other valuable traits. With respect to disease and lodging resistance, grain number per spike, and grain size and weight per unit area, it far surpassed the spring soft wheat cultivar Leningradka. A study of the progenies of individual plants of this accession showed the possibility of selecting forms with improved characteristics with respect to a number of traits. In particular, heterogeneity for brown rust resistance, length of the sprouting–heading stage, photoperiod sensitivity, and certain parameters of the spike was determined. The seeds of the best lines obtained as a result of threefold individual selection were united and gave rise to a new cultivar, Zolotoi Grebeshok. These lines were propagated and tested also individually for checking their similarity with respect to a set of traits. Field testing (2002–2003) showed that Zolotoi Grebeshok is more resistant than the initial accession to brown rust and the yield, on average, is 17.4% higher, but the cultivar heads 3 days later. There were no significant differences with respect to other morphological and quantitative traits. Cultivar testing at VIR's Pushkino branch in 2003–2005 revealed the advantage of Zolotoi Grebeshok over the spring triticale cultivar Ukro regionalized in Russia with respect to a number of important breeding traits. Since 2005 Zolotoi Grebeshok and its lines ZG-178 and ZG-186 have been undergoing ecological and production cultivar testing. Taking into account the success of the selections made and the prospects of the selection numbers, we studied the structure of the initial population and created populations with the use of protein markers.

The purpose of the present work was to explain the character of the change in the composition of gliadin biotypes during the creation of spring triticale cultivar Zolotoi Grebeshok. For this purpose we carried out an analysis of the gliadin spectra and registration in the form of protein formulas of the intrapopulation composition of Mexican-bred spring hexaploid triticale Merino/Jlo/Zebra32 (k-3503); determination and registration of the qualitative and quantitative changes in the composition of the Merion/Jlo/Zebra32 population during selection or creating the new cultivar; registration of the "protein passport" of cv. Zolotoi Grebeshok; comparative analysis of this cultivar and its individual lines and assessment of their stability during reproduction.

METHODS

We investigated the electrophoretic spectra of gliadin of individual caryopses of hexaploid triticale reproduced at VIR's Pushkino branch in 2002–2004 (designated as P-02, P-03, or P-04): the Mexican-bred acces-

†Deceased.

Content (%) of gliadin spectrum types in samples of initial triticale population Merino/Jlo/Zebra32, cultivar Zolotoi Grebeshok, and its lines

Spectrum type	Merino/Jlo/Zebra32		Zolotoi Grebeshok			ZG-53	ZG-73	ZG-97	ZG-178			ZG-186	ZG-200	ZG-205
	P-02-01	P-02-P	P-02	P-03	P-04	P-02	P-02	P-02	P-02	P-03	P-04	P-02	P-02	P-02
I	43.0	41.0	–	–	–	–	–	–	–	–	–	–	–	–
II	36.0	39.0	–	–	–	–	–	–	–	–	–	–	–	–
III	10.0	2.0	62.0	32.0	44.7	51.7	37.5	31.8	63.4	52.5	44.9	51.2	65.3	69.0
IV	4.0	4.0	34.0	54.0	45.6	45.0	62.5	60.6	30.0	40.7	28.8	47.6	32.0	29.3
V	–	2.0	–	–	–	–	–	–	–	–	–	–	–	–
VI	1.0	2.0	4.0	1.0	–	–	–	6.1	5.0	–	8.7	–	–	1.7
VII	–	1.0	–	–	–	–	–	–	–	–	–	–	–	–
VIII	–	2.0	–	–	–	–	–	–	–	–	–	–	–	–
IX	6.0	7.0	–	13.0	9.7	3.3	–	1.5	1.6	6.8	17.6	1.2	2.7	–

sion Merino/Jlo/Zebra32, k-3503, P-02 (200 caryopses); cv. Zolotoi Grebeshok, selection from k-3503, P-02, P-03, P-04 (303 caryopses); lines—selection from cv. Zolotoi Grebeshok: ZG-53, P-02; ZG-73, P-02; ZG-97, P-02; ZG-178, P-02, P-03, P-04; ZG-186, P-02; ZG-200, P-02; ZG-205, P-02 (70–100 caryopses of each line).

Isolation of proteins, electrophoretic analysis, and registration of gliadin spectra in the form of protein formulas were carried out by methods developed in VIR's Department of Biochemistry and Molecular Biology [4].

RESULTS AND DISCUSSION

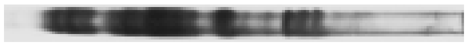
Nine types of the gliadin spectrum (figure) were revealed in the initial Merino/Jlo/Zebra32 population during an analysis of two samples (100 caryopses each). Of them, about 80% (table) are accounted for by types I and II differing only by weak component $\alpha\bar{5}$, which allows, with some share of conditionality, uniting them into one type with protein formula:

$$\alpha(\bar{5})\ 6\ \underline{7}_1\ 7_2\ \beta\ 2\ 3_2\ \underline{4\ 5}_1\ 5_2\ \gamma\ 1\ \bar{2}_1\ \underline{3}\ \bar{4}_1\ \bar{4}_2\ \omega\ \underline{2}\ \underline{3}_1\ \underline{3}\ \underline{4}\ \bar{6}_1\ \bar{7}\ \bar{8}_2.$$

Types III and IV are also similar in component composition, but differ significantly from types I and II (figure) with respect to all α-, β-, γ-, and ω-zones of the spectrum. Thus, $\alpha 7$ is present in them but not $\alpha 7_2$, a $\beta 4_1$ component appears additionally in the β zone; $\gamma\underline{5}_2$ appears in the γ zone but not $\gamma\bar{4}_2$; $\omega\bar{6}^{2}$ is identified in the ω zone instead of $\omega\bar{6}_1$. The occurrence of the type III spectrum (table) varies depending on the sample, whereas the frequency of type IV in the two samples is the same, 4%. Types III and IV of the spectrum together amount to 6–14%, which is considerably less than types I and II. Type V was detected in one of the two samples and, evidently, is found rarely in the Mexican population. It is similar in component composition to type III, but the absence of $\alpha\bar{5}$ and a very intensive $\alpha\underline{4}$ component are characteristic for it. Spectrum types II and VIII are also encountered rather rarely and according to composition are possibly hybrid. Thus, type VI includes simultaneously components characteristic for III and I, II types; type VII includes components of types IV and I, II of the spectrum; only individual components of the first four types are present in the spectrum of type VIII (figure). Blurred spectra, which can't be clearly identified, compose type IX. The process of breakdown of protein components related to germination in the spike probably occurs in triticale caryopses producing similar spectra.

Consequently, two clearly distinguishable groups of spectra (I–II and III–IV), the quantitative ratio of which with respect to average values for the two samples is about 8 : 1, can be noted in the composition of the initial Mexican accession. The remaining spectra most likely have a hybrid origin or are difficult to identify owing to blurriness related to breakdown of storage proteins.

Polymorphism for gliadin spectra is considerably lower in cv. Zolotoi Grebeshok compared with the initial Mexican population. As is seen from the data in the table, four gliadin spectrum types are found in its three samples of different reproduction years, and in samples P-02 and P-04 there are only three types each. In a quantitative respect, types III and IV predominate in Zolotoi Grebeshok, together amounting to 86–96% depending on the sample (in the Mexican population, only 6–14%). At the same time, gliadin types I and II, most characteristic for Merino/Jloa/Zebra32, are absent in cv. Zolotoi Grebeshok and in all of its lines. The relationship between type III and IV in samples of Zolotoi Grebeshok and its derivatives can change depending on

Electrophoretic spectra	Spectrum type	Protein formula			
		α	β	γ	ω
	I	$\bar{5}\ 6\ \underline{7}_1\ 7_2$	$2\ 3_2\ \underline{4\ 5_1}\ 5_2$	$1\ \bar{2}_1\ \underline{3}\ \bar{4}_1\ \bar{4}_2$	$\underline{\underline{2}}\ 3_1\ 3\ \underline{4}\ \bar{6}_1\ \bar{7}\ \bar{8}_2$
	II	$6\ \underline{7}_1\ 7_2$	$2\ 3_2\ \underline{4\ 5_1}\ 5_2$	$1\ \bar{2}_1\ \underline{3}\ \bar{4}_1\ \bar{4}_2$	$\underline{\underline{2}}\ 3_1\ 3\ \underline{4}\ \bar{6}_1\ \bar{7}\ \bar{8}_2$
	III	$\bar{4}\ 5\ 6\ \underline{7}_1\ 7$	$2\ 3_2\ 4_1\ \underline{4\ 5_1}\ 5_2$	$1\ \bar{2}_1\ \underline{3}\ 4_1\ \underline{5_{\underline{2}}}$	$\underline{\underline{2}}\ \underline{3_1}\ \underline{3}\ \underline{4}\ \bar{6}_2\ \bar{7}\ \bar{8}_2$
	IV	$\bar{5}\ 6\ 7_1\ 7$	$2\ 3_2\ 4_1\ \underline{4\ 5}_1\ 5_2$	$1\ \bar{2}_1\ \underline{\underline{3}}\ \bar{4}_1\ 5_2$	$\underline{\underline{2}}\ \underline{3_1}\ \underline{3}\ \underline{4}\ \bar{6}_2\ \bar{7}\ \bar{8}_2$
	V	$\underline{4}\ \underline{6}\ 7_1\ 7$	$2\ \underline{3_{\underline{2}}}\ 4_1\ \underline{4\ 5_1}\ 5_2$	$1\ \bar{2}_1\ \underline{\underline{3}}\ 4_1\ 5_2$	$\underline{\underline{2}}\ \underline{3_1}\ \underline{3}\ \underline{4}\ \bar{6}_2\ \bar{7}\ \bar{8}_2$
	VI	$\bar{4}\ 5\ 6\ 7_1\ \bar{7}\ 7_2$	$2\ \underline{3_{\underline{2}}}\ \bar{4}_1\ \underline{4\ 5_1}\ 5_2$	$1\ 2_1\ \underline{\underline{3}}\ \bar{4}_1\ 5_2$	$\underline{\underline{2}}\ \underline{3_1}\ \underline{3}\ \underline{4}\ \bar{6}\ \bar{7}\ \bar{8}$
	VII	$5\ 6\ 7_1\ 7\ 7_2$	$2\ 3_2\ 4_1\ \underline{4\ 5_1}\ 5_2$	$1\ 2_1\ \underline{3}\ \bar{4}_1\ \bar{4}_2\ 5_2$	$\underline{\underline{2}}\ \underline{3_1}\ \underline{3}\ \underline{4}\ 6_2\ \bar{7}\ 8_2$
	VIII	$6\ \underline{7}_1\ \bar{7}\ \bar{7}_2$	$2\ 3_2\ 4_1\ \underline{4\ 5}\ 5$	$1\ \bar{2}_1\ \underline{3_1}\ \underline{3}\ \bar{4}_1\ \bar{5}_2$	$2\ \underline{3_1}\ \underline{3}\ 4\ \bar{6}\ \bar{7}\ \bar{8}_2$
	IX	Blurred spectra			

Gliadin spectrum types revealed in the composition of the triticale accession Merino/Jlo/Zebra32 and cultivar Zolotoi Grebeshok and their protein formulas

the year of reproduction, but all in all their quantity is not less than 85% (except line ZG-178 in 2004). This index is a characteristic trait of cv. Zolotoi Grebeshok. Since the component composition of types III and IV of the gliadin spectrum is very similar (differences only with respect to weak component $\alpha\bar{4}$), in the "protein passport" of cv. Zolotoi Grebeshok we can enter the obligatory presence of at least 85% genotypes marked by gliadin spectra with protein formula:

$$\alpha\ (\bar{4})\ 5\ 6\ 7_1\ 7\ \beta\ 2\ 3_2\ 4_1\ \underline{4\ 5_1}\ \underline{5_2}$$

$$\gamma\ 1\ 2_1\ \underline{3}\ 4_1\ 5_2\ \omega\ \underline{2}\ \underline{3_1}\ \underline{3}\ \underline{4}\ \bar{6}_2\ 7\ \bar{8}_2.$$

In addition to the main (III and IV) types of spectrum, in samples of Zolotoi Grebeshok P-03 and P-04 we revealed respectively 13 and 9.7% blurred spectra difficult to identify, which is probably related to germination of grain in the spike. Such spectra were absent in the sample taken in 2002 favorable for the formation and filling of grain. The relationship between the main types of spectrum also varied depending on reproduction conditions. Thus, the ratio of spectra of types III : IV was about 1.8 : 1 in 2002, 0.6 : 1 in 2003, and 0.98 : 1 in 2004.

All Zolotoi Grebeshok lines keep the traits characteristic for the given cultivar (table): 2–4 gliadin types. The most characteristic types III and IV of the spectrum amount from 3.7% in ZG-178 (P-04) to 100% in ZG (P-02). The relationship between biotypes III and I differs in various lines depending on the reproduction year. The number of unidentified blurred spectra also varies from 17.6% in G-178 (P04) to absent in Zolotoi Grebeshok (P-02), ZG-73 (P-02), and ZG-205 (P-02). Hybrid spectra were also found in the composition of individual lines: the maximum number 6.1% in ZG-97 (P-02) and 5.0 and 8.78% respectively in ZG-178 (P-02, P-04).

The initial Mexican accession and lines selected from it have stably high fertility of flowers and propagate mainly by self-pollination. Even without isolation they retain phenotypic homogeneity well. At the same time, single taller plants—spontaneous hybrids—appear in their crops. Evidently, precisely the F_1 hybrid caryopses setting as a result of cross-pollination are marked by the "hybrid" spectra revealed in the present investigation. However, it is difficult to presume that the change from year to year in the relationship between gliadin spectrum types I and II in the initial accession and types III and IV in Zolotoi Grebeshok and its lines is related to substantial genotypic changes in the composition of the populations. This phenomenon is probably due to the effect of environmental conditions on the occurrence of the fifth and fourth components in the α-zone, with respect to which the spectrum types differ: I from II and III from IV.

Thus, marking gliadin of triticale genotypes by spectra made it possible to reveal intrapopulation polymorphism in the initial Mexican Merino/Jlo/Zebra32 population and to show that purposeful selection of biotypes having a set of economically valuable traits under conditions of northwestern Russia led to a substantial change in the structure of this initial population. Genotypes marked by spectra of gliadin types III and IV (figure) with a low frequency of occurrence in the Mexican population compose the base of cv. Zolotoi Grebeshok. They are the most adapted and highly productive under conditions of the region. The presence of such gliadin biotypes in a number not less than 85% is a stable and characteristic trait, a unique “protein passport” of the new triticale cultivar Zolotoi Grebeshok. Registration of the cultivar according to the protein passport gives additional information for legal protection and also allows reliable checking of the purity and authenticity of the cultivar at all stages of seed production and revealing possible changes in its structure in farm-scale crops.

The results of the investigations indicate the need for maximum use of the potential of triticale accessions from VIR’s world collection and new hybrid combinations for selection under various environmental conditions for the purpose of creating cultivars most adapted and productive for various regions of Russia. An effective way to achieve this goal is the organized exchange of hybrids of early generations between breeding establishments and the use of molecular and, in particular, protein markers as the most accessible for identifying individual biotypes, analyzing the structure of the initial population, determining its dynamics during breeding, and preserving the authenticity and purity of the newly created cultivars.

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