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Original scientific paper

GLIADIN AND GLUTENIN POLYMORPHISM IN DURUM WHEAT LANDRACES AND BREEDING VARIETIES OF AZERBAIJAN

Hamlet Baykishi SADİGOV

Genetic Resources Institute of ANAS, Baku, Azerbaijan

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Durum wheat genotypes including 7 landraces and 17 breeding varieties were studied. Polyacrylamide gel electrophoresis under acidic conditions of pH 3.1 was used to study gliadin and glutenin polymorphisms. In total, 32 gliadin and 8 high molecular weight glutenin alleles were identified. The contribution of B genome (58.5%) to the allelic variation of durum wheat varieties was higher than of A genome. The cluster analysis delineated genotypes into four main clusters. According to cluster analysis, legitimacy identifying the distribution of botanical varieties through the tree was observed. The study confirms the suitability of biochemical markers for cultivar identification and genetic relation study in durum wheat genotypes.

Key words: stock proteins, durum wheat, landrace, electrophoresis, botanical variety

INTRODUCTION

Since the end of the twentieth century, the prospects of the use of protein and DNA markers to study the extent of genetic variation in different plant species have been widely investigated (BABAYEVA *et al*., 2009; IZZATULLAYEVA *et al*., 2014). Although protein markers have been supplanted by DNA markers in many cases and various molecular methods are regularly used as breeding tools in wheat (ALIYEV *et al*., 2007), protein markers, like seed storage proteins (gliadins and glutenins) remain highly effective for wheat breeding purposes as genetic markers (ZHELEVA *et al*., 2007). Genetic identification of plant genotypes using protein markers is more easear, time and cost effective in comparison with the DNA markers (DVOŘÁČEK and ČURN, 2003). Gliadins and glutenins which form up to 80% of total wheat endosperm protein reflect with more accuracy the genotypes, independently from the environmental effects (AUTRAN *et al*., 1995) and therefore are used in identifying cultivar, registration of new varieties, classification of crop species and in studying phylogenesis and genetic diversity, thereby improving the efficiency of

Corresponding author: Hamlet Baykishi SADİGOV,Genetic Resources Institute of ANAS, Avadlig ave. 155, AZE 1106, Baku, Azerbaijan,
E-mail: hamlet.sadiqov@yahoo.com

wheat breeding programs in cultivar development (CİAFFİ *et al*., 1999; GIANIBELLI *et al*., 2001; NAGHAVI *et al*., 2009; POPERELYA, 1996; SOZINOV, 1985).

Seed storage proteins are also involved in the determination of bread and pasta-making quality, in bread and durum wheat respectively (KONAREV, 1983). Polymorphism, inheritance, linkage with quantitative and qualitative traits of gliadin and glutenincoding loci of durum wheats are not studied well in compare with bread wheats (KUDRYAVCHEV, 1994).

In durum wheat gliadincoding loci (Gld 1A, Gld 1B, Gld 6A, Gld 6B) are placed on the short arms of group 1 and 6 chromosomes and glutenincoding loci (Glu 1A and Glu 1B) on the long arms of group 1 chromosomes (AHMADOV *et al*., 2003; DU CROSS *et al*., 1983; KUDRYAVCHEV, 1994; SADİGOV, 1994; SOZİNOV, 1985). Localization of these proteins has been revealed by two-dimensional electrophoretic analysis in aneuploid lines of durum wheat variety Langdon. Electrophoretic spectra of gliadincoding loci are conditionally divided into ω-, γ-, β-, and α-zones according to the migration in the gele (YİLDİRİM *et al*., 2011). Molecular weight range of gliadin components in α-, β-, γ- zones is 11400 - 57300, and in ω-zone 64000-73000 dalton (BİETZ and WALL, 1980; CHARBONNİER, 1974; SOZİNOV, 1985).

In ω-zone of gliadin electrophoregrams mainly localized spectra controlled by allele genes of 1A and 1B chromosomes, in γ-zone 1B, 6B, in β-zone 6B and in α-zone by allele genes of 6A chromosome. Electrophoretic components of gliadin and glutenin during one-dimensional electrophoresis in acidic conditions (A-PAGE, pH 3.1) are moving as linked groups or blocks. In F_1 progeny allele components of gliadin and glutenin have been confirmed to be inherited codominantly, while in F_2 generation Mendelian 1: 2: 1 ratio was observed. These electrophoretic componenets which are controled by gene clusters are not dependent on the enviromental conditions and inherited as a linked block from generation to generation. Moreover, allele genes of gliadin and glutenincoding loci are localized in the same linkage group with quantitative and mostly qualitative traits and can be considered as genetic markers of these traits (KARİMOV, 2012; KUDRYAVCHEV, 1994; METAKOVSKİY *et al*., 1990; POPERELYA, 1989; SADİGOV, 2013).

The aim of our study was evaluation of polymorphism of gliadin and glutenin loci and determination of genetic relationships among durum wheat landraces and breeding varieties of Azerbaijan.

MATERIALS AND METHODS

In this study 25 durum wheat (*T.durum* Desf.) genotypes were analysed: 7 landraces (Gara bughda, Sary bughda, Shirvan, Agh bughda, Yerly Garagichig, Aran deni and Bozakh) and 17 breeding varieties (Mirbashir 50, Tartar, Tartar 2, Garagilchig 2, Kahraba, Barakatly 95, Turan, Sharg, Jafary, Sevinj, Mughan, Shiraslan 23, Vugar 80, Alinja 84, Gyzil bughda, Mirvary and Garabagh) from the Azerbaijan Farming Institute and from Genetic Resources Institute of Azerbaijan National Academy of Sceinces. Studied varieties belonged to botanical varieties var. *leucomelan,* var. *provinciale,* var. *leucurum,* var. *horanoleucurum,* var. *hordeiforme,* var. *melanopus,* var. *apulicum* and var. *alboprovinciale*. Langdon (*T. durum* Desf.) variety were used as a reference cultivar (KUDRYAVCHEV, 1994; SADİGOV, 1994). Two grains (each from one spike) of each genotype were tested. Storage proteins were extracted with 70% ethanol from a whole wheat grain. One-dimensional polyacrylamide gel electrophoresis (A-PAGE) (ph 3.1) was done according to the modified method of POPERLYA (1989). The gel solution contained 8% acrylamide, 0.4% methylenebisacrylamide, 0.1% ascorbic acid and 0.001% $Fe_2(SO_4)_3$ x $7H_2O$. PAGE was carried out in a 0.005 M glysine asetat buffer solution (pH 3.1) for 4 h at a constant voltage of 450

V. After the electrophoresis, the gel was fixed with 10% trichloroacetic acid (TCA) for at least 20 min and stained overnight in a solution containing 0.04% Coumassie R-250 and 10% TCA. The allelic variants of gliadin component blocks were identified and designated according to the catalogue compiled earlier (KUDRYAVCHEV, 1994; SADIGOV, 1994).

Band presence (1) and absence (0) were recorded for all genotypes and the data were subjected to tree clustering using SPSS software (SPSS, 2003). Cluster analysis was done based on the JACCARD'S similarity coefficients (1908).

RESULTS AND DISCUSSION

In present study, A-PAGE of grain proteins was performed in order to investigate gliadin and glutenin polymorphism among durum wheat genotypes. Durum wheat landraces and breeding varieties showed various banding pattern using A-PAGE technique. Electrophoregram of different wheat varieties and detected alleles are given in Figure 1 and in Table 1.

Table 1. Gliadin and Glutenin alleles of durum wheat landraces and breeding varieties

№	**Varieties**	**Botanical varieties**	**Gli alleles**				**Glu alleles**	
			1A	**1B**	**6A**	**6B**	**1A**	**1B**
1	Gara bughda	(v. *leucomelan*)	16	17	4	4	0	5
2	Sary bughda	(v. *leucurum*)	15	9	4	3	0	3
3	Shirvan bughda	(v. *hordeiforme*)	16	9	1	10	2	2
4	Agh bughda		15	16	3	11?	0	1
5	Yerly Garagilchig	(v. *melanopus*)	15	9	6	3	0	1
6	Aran deni	(v. *apulicum*)	15	9	5	11?	0	1
7	Bozakh	(v. *hordeiforme*)	16	18	1	4	0	1
8	Sharg	(v. *horano-leucurum*)	12	12	5	2	0	3
9	Garagilchig 2	(v. *apulicum*)	3	3	5	1	0	5
10	Barakatly 95	(v. *hordeiforme*)	3	14	3	1	0	2
11	Vugar 80	(v. *leucurum*)	3	3;17	2	6	2	2
12	Jafary	(v. *horano-leucurum*)	12	12	5	2	0	3
13	Sevinj	(v. *hordeiforme*)	13	14	4	4	0	1
14	Gyzil bughda	(v. *hordeiforme*)	13	15	3	9	0	1
15	Langdon (reference)		3	3	1	1	0	4
16	Kahraba	(v. *leucurum*)	3	14	4	11	0	2
17	Tartar	(v. *provinciale*)	13	12	6	6	0	2
18	Tartar 2	(v. *albo-provinciale*)	15	9	9	4	0	2
19	Turan	(v. *leucomelan*)	3	19	5	8	0	2
20	Mirbashir 50	(v. *leucurum*)	14	17	4	10	0	2
21	Alinja 84	(v. *leucurum*)	3	14	4	6	0	2
22	Shiraslan 23	(v. *leucurum*)	3	17	3	2	1	2
23	Mirvary	(v. *leucurum*)	15	9	3	3	0	3
24	Garabagh	(v. *provinciale*)	16;18	9;17	1	12?	0	2
25	Mughan	(v. *leucurum*)	3	17	4	2	0	2

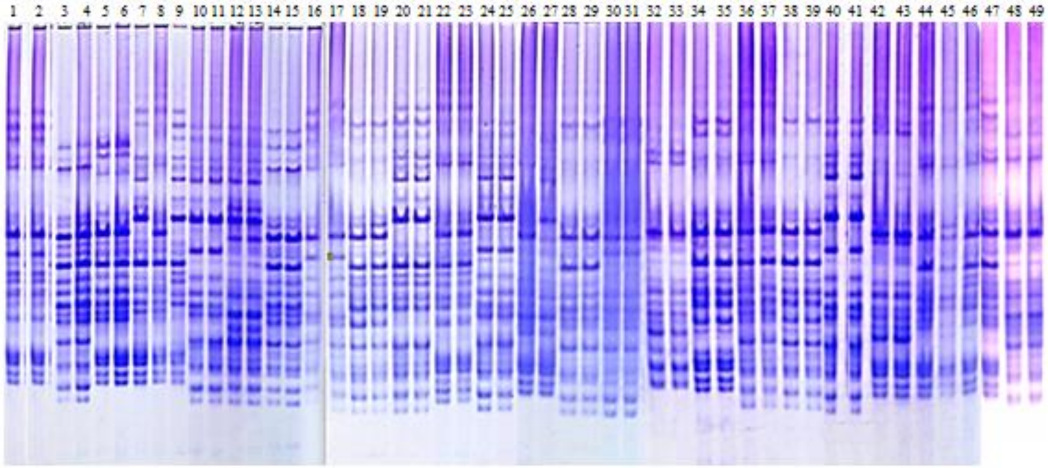

Fig 1. A-PAGE showing banding pattern of gliadins in the durum wheat landraces and breeding varieties. 1-2 Barakatly 95; 3-4 Sary bughda; 5-6 Mirvary; 7-8 Vugar 80; 9 Langdon; 10-11 Jafary; 12-13 Tartar; 14-15 Gara bughda; 16-17 Kahraba; 18-19 Shirvan bughda; 20-21 Garagilchig 2; 22-23 Turan; 24-25 Sharg; 26-27 Garabag; 28-29 Bozakh; 30-31 Sevinj; 32-33 Mirbashir 50; 34-35 Agh bughda; 36-37 Mughan; 38-39 Yerly Garagilchig; 40-41 Aran deni; 42-43 Gyzil bughda; 44-45 Shiraslan 23; 46-47 Alinja 84; 48-49 Tartar 2.

This study confirmed the highest allelic variability at gliadin loci compared to variability of alleles encoding high molecular weight glutenins (HMW- GS) in the investigated set of wheat genotypes. In total, 32 gliadin and 8 HMW-GS alleles were identified (Fig. 3). The obtained results are comparable to the previous reports of DVOŘÁČEK et al. (2013). The gliadin allele Gld 1A 3 showed the highest frequency. In the cases of HMW-GS the highest occurrences was found for Glu 1A 0. Some alleles were rare. Gli 1A 14, Gli 1A18, Gli 1B16, Gli 1B18, Gli 1B15, Gli 1B19, Gli 6A 9, Gli 6B 9, Gli 6B 8, Gli 6B 12 and Glu 1B 4 were found in only one genotype. In general, there was more variation for Gli 6B and Glu 1B loci in evaluated material as compared to other Gli and Glu loci. The contribution of B genome (58.5%) to the allelic variation of durum wheat varieties was higher than of A genome.

In the present study Glu 1A locus of A genome contributed 3 alleles (null, 1 and 2), where the Null allele encoding no subunit had prevalence and 1 was found in only one variety Shiraslan 23. Using A-PAGE technique, durum wheat varieties grown in Syria were all found to carry the null allele of Glu 1A (MIRALI *et al*., 1999; YAN *et al*., 2007). On contrary, TABASUM *et al.* (2011) have mentioned the dominancy of 2* and 1 allele in a set of 76 bread wheat genotypes. Five alleles were detected at Glu 1B locus. Previously, 7 alleles were reported by other authors (TABASUM *et al*., 2011) in bread wheat.

Allelic variants of gliadin loci are used as genetic markers for adaptation to certain environmental conditions, resistance to abiotic stress factors etc. For instance, Gli 1B 3 allele, which was noted in Garagilchig 2, Vugar 80 and reference variety Langdon was used as a marker for the 1B/1R translocation. This translocation has favorite effects mainly on disease resistance, grain yield and biomass accumulation, therefore, has been utilized for durum wheat breeding.

Alleles Gli 6B 11 in Agh bughda and Aran deni and Gli 6B 12 in Garabagh variety need to be defined more precisely thorough hybridization and analyzing in F_2 generation.

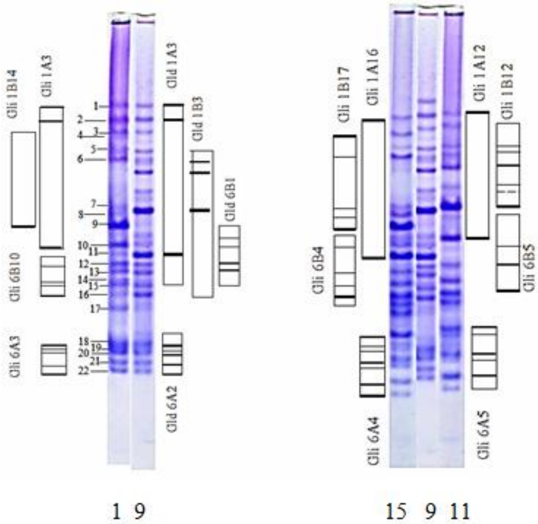

Fig 2. Representative profiles for identification allele component blocks in some varieties in compare with reference variety Langdon. 1 Barakatly 95; 9 Langdon; 11 Jafary; 15 Gara bughda.

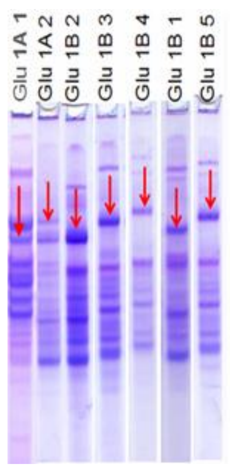

Fig 3. A-PAGE profile for HMW-GS alleles identified in durum wheat landraces and breeding varieties.

Among the 25 varieties studied, 23 were homogenous and 2 were heterogeneous. Intra-varietal heterogeneity was observed in Vugar 80 and Garabagh varieties. In Vugar 80 Gli 1B locus exhibited alleles Gli 1B 3 and Gli 1B 17. Two biotypes with two different alleles at Gli 1A (Gli 1A 16 and Gli 1A 18) and two alleles at Gli 1B (Gli 1B 9 and Gli 1 B17) were found within Garabagh variety. The rest varieties were uniform for the gliadin and HMW - GS patterns.

The heterogeneity in Vugar 80 and Garabagh varieties is acceptable and was also noted in previous experiments (SADIGOV, 2013). This can be due to the breeding programs in former USSR countries which were did not aim at creating completely homogeneous varieties. Selection in the early generations without applying the pedigree method may lead to some variability. Several researchers believe that the intra-varietal heterogeneity provides opportunities for improving the variety (BELDOROK *et al*., 2000). Thus, a variety consisting of different biotypes provides more stable yields in changeable environmental conditions, due to a compensation of biotype-environment interaction. Genetically different biotypes of the same variety are affected differently by the environment during multiplication and by storage conditions. Therefore, according to STOYANOVA (1996) each genotype identified heterogeneous for gliadin spectra should be multiplied and stores as an individual accession.

Electrophoretic pattern of gliadins and glutenins enabled to distinguish almost all landraces and breeding varieties from each other, except Sharg and Jafary. To give an example, gliadin and glutenin pattern of Barakatly 95 variety was as follows: Gli 1A 3, Gli 1B 14, Gli 6A 3, Gli 6B 1, Glu 1A 0 and Glu 1B 2. Electrophoretic components controlled by allele genes responsible for the gliadin synthesis were inherited as a linked group (block). In Barakatly 95, which was compared with Langdon variety 1, 2, 9 components were encoded by Gli 1A 3 allele component block localized on 1A chromosome (Fig. 2). While 3, 4, 8 intensive and 6, 7 minor components were encoded by Gli 1B 14 allele component block localized on 1B chromosome. Genetic control of 11, 14 minor and 12, 15, 16 intensive components was provided by Gli 6B 1 allele component block localized on 6B chromosome. Gli 6A 3 allele component block was responsible for the 18, 19, 20, 21, and 22 electrophoretic components. Two alleles Null and 2 were identified at Glu 1A and Glu 1B loci of Barakatly 95 respectively.

The frequency of gliadin coding loci differed in landraces and in breeding varieties. So, the frequency recorded for the alleles of Gli 1A and Gli 1B loci was lower in breeding varieties than in landraces, while Gli 6A and 6B allele component blocks had equal frequencies in both type of varieties.

Twenty five varieties were also subjected to UPGMA cluster analysis to get an idea on genetic relationship among the studied genotypes. The results are given in Figure 4. The cluster analysis delineated genotypes into four main clusters. The first and the third clusters contained only two genotypes, while the other two clusters were further divided into subgroups. The varieties Sharg and Jafary in the first cluster showed 100% similarity and were almost identical in their electrophoregrams. They belonged to the same botanical varieties *horanoleucurum*. The second cluster combined 2 subclusters: one containing 6 varieties out of which 4 were landraces, the second composed of 4 genotypes with 2 landraces (Gara bughda and Bozakh) being closer to each other. In general, 6 of 7 landraces involved into the experiments fall into cluster II. The last landrace, namely Shirvan bughda was included into third cluster together with heterogeneous variety Garabagh. The landrace was closer to biotype containing Gli 1A 16 and Gli 1B 9 alleles. The another biotype of Garabagh which contained rare alleles for Gli 1A, Gli 6B loci was quite differ from Shirvan bughda and other genotypes and in homogeneous state could form a separate cluster. The fourth cluster contained 2 subgroups with 2 and 9 breeding varieties respectively.

According to cluster analysis, legitimacy identifying the distribution of botanical varieties through the tree was observed. Thus, from 8 var. *leucurum* varieties 6 fall into same subgroup in cluster IV and 2 (Sary bughda and Mirvary) into subgroup of cluster II. Sary bughda and Mirvary were genetically very close and differed only for Gli 6A locus. Similar regularity was noted also for 2 *horanolecurum* varieties forming cluster I and 3 out of 5 *hordeiforme* varieties which grouped in the same subgroup of cluster II. Botanical varieties *apulicum*, *leucomelan* and *provinciale* were distributed among clusters. This fact may also be due to the low number of genotypes included into experiment from these botanical varieties.

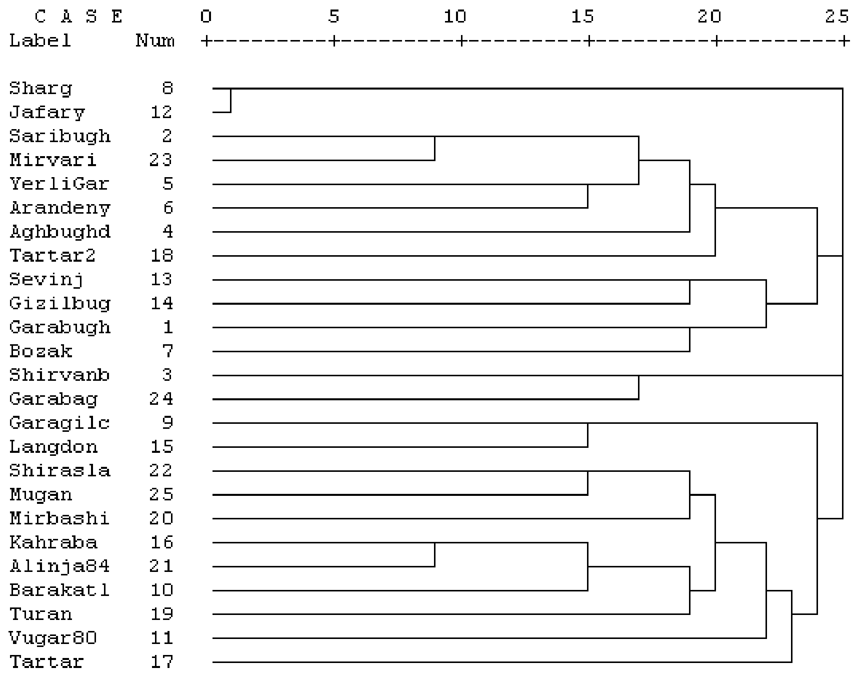

Fig 4. Dendrogram showing the genetic relationship among 25 durum wheat landraces and breeding varieties.

Both some legitimacy and irregularity were observed regarding to the correspondence between pedigrees and the position of the genotypes in the tree. The situation of having accordance between the pedigrees and grouping into clusters was recorded for all breeding varieties originated from crossing with Sharg variety. Thus varieties Shiraslan 23, Alinja 84, Mirbashir 50, Vugar 80 and Barakatly 95 were assembled in one subgroup of cluster 4, which confirm the existence of common alleles for gliadin and glutenin loci of these genotypes. However, genotypes Shiraslan 23 and Vugar 80 got from same cross (Sharg x Oviachik 65) were less related in the dendrogram. This may be due to the fact that these genotypes were selected to have different morphological characteristics. It can be also partially explained by heterogeneous nature of Vugar 80 variety. No linkage between pedigree and grouping was observed for several genotypes, namely, Tartar 2 and Garabag originated from cross with Tartar; and Garagilchig 2 and Yerly Garagilchig. On the other hand, some varieties with similar allelic composition (and therefore related in the dendrogram) were unrelated in their pedigrees. Their similarity can be either due to heterogeneity of these varieties or false pedigrees.

So, seed storage protein electrophoregrams can be used as a tool to reveal genetic relationship among durum wheat genotypes, however, many factors, such as heterogeneity, false pedigrees etc. should be taken into account when drawing any conclusion. The genotypes estimated as genetically diverse based on cluster analysis and with good quality subunits could be combined through different breeding procedures for the production of high quality wheat. The present study determines gliadin and glutenin allele composition of Azerbaijani durum wheats and succeeded to distinguish almost all analysed varieties from each other. This confirms the suitability of biochemical markers for durum wheat cultivar identification. Electrophoretic evaluation of gliadin and glutenin alleles can contribute to the varietal authenticity by providing legal protection of registered durum wheat varieties

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POLIMORFIZAM GLIADINA I GLUTENINA U DOMAĆOJ DURUM PŠENICI I OPLEMENJIVANJE SORATA AZERBEJDŽANA

Hamlet Baykishi SADİGOV
İnstıtut za genetiče resurse ANAS, Baku, Azerbedžijan

Izvod

Vršena je genotipizacija 7 domaćih sorata i 17 genotipova durum pšenice. Korišćen je metod PAGE u kiselim uslovima sredine. Ukupno je identifikovano 32 alela gliadina i 8 alela visoke molekularne težine glutenina. Doprinos B genoma (58,5 %) alelnom variranju durum sorata je bio veći od doprinosa genoma A. Klaster analizom izvršeno je grupisanje u četiri glavna klastera. Prema klsater analizi utvrđena je identifikacija distribucije botaničkih varieteta. Rezultati potvrđuju pogodnost biohemijskih markera za identifikaciju kultivara i ispitivanje genetičkog odnosa durum genotipova.

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