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Genetic determination of technological quality in *Triticum durum*

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Abstract

Background and Purpose: This paper presents the results of an investigation of gliadin alleles variability and their relationship to technological quality components in 21 durum wheat cultivars.

Material and Methods: Seeds of 21 durum cultivars were crashed and used for extraction of gliadins by 70% ethanols. At least 30 seeds were used for gliadin extraction. The composition of gliadin components was analyzed by acid polyacrylamide gel electrophoresis (pH=3.1). Electrophoregrams were used for identification of gliadin alleles. Technological quality of geneticaly divergent durum cultivars were evaluated by determination of wet gluten content and rheological (farinographic) dough properties.

Results and Conclusion: Polymorphysms of alleles at each locus was registered. At four gliadin loci 27 different alleles were determined by analysis of 21 durum cultivars. Each cultivar had different gliadin allele composition. Frequency of each allele was computed and varied in the ratio from 4.8% to 42.9%. Values of flour water absorption and gluten contents had on the level of B_1 and B_2 quality classes. Gliadin alleles at the *Gli-B1* locus showed the highest positive connection with gluten contents. High frequency of alleles was related with good gluten quality and water absorption of flour. Gliadins can be used as a marker for biological traits of wheat.

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INTRODUCTION

Triticum durum is a tetraploid species with two diplod genomes AA and BB. Each of these genomes has 7 pairs of chromosomes (n=14 and 2n=28 chromosomes). Durum wheat is important for human food which is used for making pasta, bread, and related products are associated with medium to high protein contents and compositions. Many investigations have been focused on variability of storage proteins and their impact on technological quality parameters both in durum and bread wheat *(1, 2)*.

Storage proteins represent products of numerous alleles of *Gli* loci which are in *Triticum aestivum* wheat located at the short arm of 1A, 1B, 1D, 6A, 6B and 6D chromosomes *(3, 4)*. In durum wheat *Gli-A1* and *Gli-B1* loci are located at the short arm of 1. and *Gli-A2* and *Gli-B2* are located at the short arm of 6. homologous chromosomes *(5)*. For each locus multiple allelism were identified.

TABLE 1

The frequency of alleles at the *Gli-1* and *Gli-2* loci.

Gli-A1 locus		*Gli-B1 locus*		*Gli-A2 locus*		*Gli-B2 locus*	
Allele	Frequency %	Allele	Frequency %	Allele	Frequency %	Allele	Frequency %
b	4.76	*a*	42.86	*c*	19.05	*h*	4.76
h	23.80	*c*	33.33	*k*	9.52	*a*	38.09
a	23.80	*b*	9.52	*g*	14.28	*c*	14.28
e	14.28	*d*	14.28	*n*	9.52	*g*	4.76
c	33.33			*j*	4.76	*o*	4.76
				e	9.52	*b*	9.52
				d	4.76	*d*	9.52
				b	23.8	*i*	4.76
				f	4.76	*e*	9.52

TABLE 2

Gli- allele composition and quality parameters in durum wheat.

Cultivar	Gli –allels				Wet gluten (%)	Dry gluten (%)	Water absorption (%)	Quality number	Quality group
	A1	*B1*	*A2*	*B2*					
YG-2313	*b*	*a*	*c*	*h*	11.662	4.535	68.5	56.0	B_1
YG-2591	*h*	*c*	*c*	*a*	39.615	11.435	63.6	50.4	B_2
YG-3183	*h*	*c*	*k*	*a*	44.790	14.135	63.6	63.1	B_1
YG-4541	*a*	*a*	*g*	*c*	36.230	10.700	60.4	51.4	B_2
YG-5141	*a*	*a*	*n*	*a*	39.630	11.515	65.0	52.8	B_2
YG-5249	*e*	*b*	*j*	*a*	42.105	12.512	68.2	62.4	B_1
YG-5257	*c*	*a*	*c*	*c*	20.110	7.032	62.5	48.6	B_2
YG-5267	*c*	*a*	*c+a*	*g*	42.100	12.105	64.7	48.4	B_2
YG-5708	*a*	*a*	*e*	*o*	30.695	9.390	65.0	52.6	B_2
YG-6281	*h*	*b*	*k+f*	*c*	38.880	10.735	65.8	56.2	B_1
YG-6755	*c*	*c*	*d*	*b*	27.305	8.277	64.9	57.7	B_1
YG-3709	*e*	*a*	*b*	*d*	26.615	8.810	66.3	57.5	B_1
YG-4682	*h*	*d*	*f*	*I*	32.105	9.817	64.6	56.4	B_1
YG-5251	*c*	*d*	*b*	*d*	31.962	11.430	66.4	56.2	B_1
YG-6934	*a*	*c*	*g*	*e*	10.390	3.845	57.1	0.0	C_2
YG-7154	*a*	*c*	*g*	*e*	38.977	11.177	63.8	62.6	B_1
YG-7160	*e*	*a*	*e*	*a*	36.740	11.410	64.2	51.8	B_2
YG-7164	*c*	*c*	*n*	*b*	40.710	13.007	65.6	56.2	B_1
YG-7578	*h*	*d*	*b*	*a*	32.245	10.610	58.8	54.5	C_1
YG-9052	*c*	*c*	*b*	*a*	38.567	12.205	65.4	56.4	B_1
YG-9674	*c*	*a*	*b*	*a*	36.485	12.215	64.8	45.1	B_1

The polymorphysms of *Gli* alleles play an important role in increasing genetic variability *(6, 7)*. Gliadins which are separated on gel by electrophoresis showed high variability and can be used for establishing genotype variability. Electrophoregrams obtained by this method are very suitable for allele identification on the basis of gliadin components which differ according to their number, mobility and color intensity *(8)*. Components or block of components is under the control of corresponding allele. By using this method many wheat cultivars have been identified *(9, 10)*.

Gliadin and glutenins are the main components of storage proteins and have a positive influence on gluten quality. The presence or absence of some components related to gluten properties, i.e. dough strength and elasticity. Viscoelasticity of gluten is the most important quality parameter of pasta quality from durum wheat flour. This trait is connected to certain protein components of γ-gliadins and LMW glutenins which are controlled by multiple allele at the *Gli-B1* locus *(11, 12)*. Relationships between gliadin and gluten elasticity, as well glutenins and viscosity were studied by *(13)* and positive

correlation was found. Some investigations indicated a positive connection between single band 42-γ and weak gluten as well as 45-γ components and strong gluten in durum wheat *(1, 14, 15)*. This is very important for breeding of durum wheat and predicting the quality of flour and dough.

The aim of this study was to establish *relationship* correlation between particular gliadin components and technological quality parameters.

MATERIALS AND METHODS

By electrophoretic analysis of gliadins, 21 cultivars of *Triticum durum* were examined. Extraction and separation of gliadins was carried out according to Novoselskaya *et al. (16),* by poliacrilamid gel electrophoresis at pH 3.1. 8.33% polyacrylamide gel was used, prepared with: 12.5 g acrilamid, 0.62 g N,N'-methylene-bis--acrylamide, 0.15 g ascorbic acid, 200 µl 10% ferro-sulfate heptahydrate, diluted in 150 ml Al-lactate buffer (pH 3.1). Polymerisation of gel was initiated by 10 µl 3% hydrogen peroxide. The prepared solution was poured into the vertically oriented apparatus, where gels were formed between glass plates (dimension 150 × 150 × 1.8 mm). Sites for application of samples were formed with a special comb, whose cogs were immersed in solution before polymerisation.

Gliadins were extracted from whole kernel by 70% ethyl alcohol. From each cultivar 20 µl of extract was applied to the gel. Besides the analyzed samples, extract of gliadins of cultivars Bezostaya, Langdon and Insignia, were used as universal standards.

Separation of the gliadin molecules was performed during 2.5 to 3 hours, in an electric field under constant voltage of 550 V and in 5 mÌ aluminum lactate buffer. At the beginning of the analysis , temperature of the electrophoretic buffer was 10° C, while at the end it was 25-30° C.

After electrophoresis, gels were immersed for 15 minutes in 300 ml of a fixative, and then stained in alcohol solution 0.05% Coomassie Briliant Blue R-250, and 250 ml 10% threechloroacetic acid added. The following day, solution of stain was poured off. Gels were washed in water and photographed. Determination of gliadin block for each cultivar was done on the basis of comparison with electrophoregrams of standard cultivars (*Bezostaya, Langdon, Insignia*).

For gluten content (Kaludjerski and Filipovic, 1998) *(17)* determination 10 g of flour were mixed with 5 ml of 2 % NaCl. The obtained dough ball should be washed in water. Contents of wet gluten computed by formula: wet gluten (%) = a x 100/m (a = mass of wet gluten, m = mass of sample measured in grams). Dry gluten was obtained by wet gluten drying at 105^0C. Dough rheological properties were analyzed by Brabender farinograph (ICC Standard No 115/1). By this analysis water absorption and cultivar quality number and class were estimated.

RESULTS AND DISCUSSION

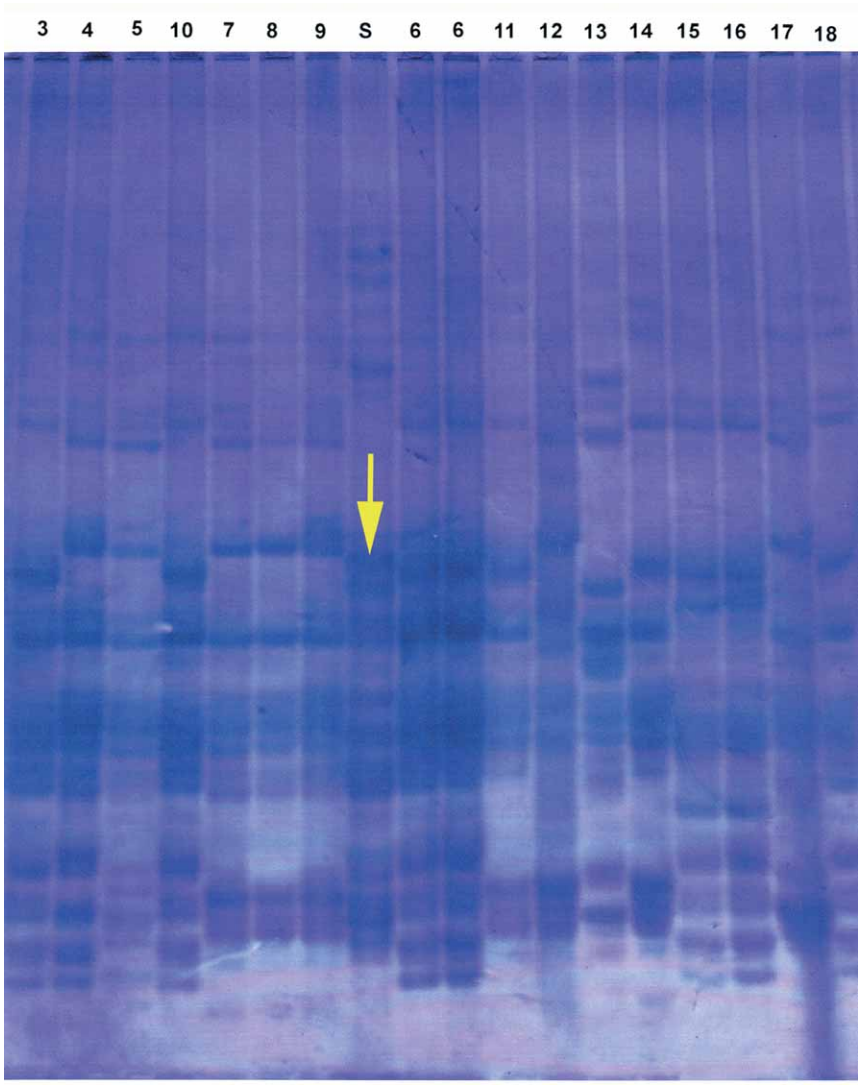
Identification of 21 cultivars of *Triticum durum* were carried out by using gliadin electrophoregrams obtained by electrophoresis. Gliadin electrophoregrams represent «finger prints« of wheat cultivars. The obtained electrophoregrams for the analyzed cultivars of durum wheat differed in respect to the presence of some components. For each cultivar the electrophoregram was specific, and the presence of gliadin components varied in ratio from 18 to 28. Similar data on the number of bands separated by one-dimensional electrophoresis were found by the following authors: Knezevic *(6)* (range of gliadin components from 22 to 30 gliadin components per cultivar); Brown and Flavell *(18)* (15 to 30 gliadin components per cultivar). However, Ram *et al. (19)* identified 147 bands by acid PAGE analysis of gliadin from 159 Indian wheat cultivars.

One band or block of bands is the phenotypic expression of gliadin components inherited under the control of gliadin allels. The gliadin blocks differed according to the number of components, their color intensity and relative mobility. Determination of gliadin alleles in the analyzed durum wheat was conducted on the basis of numerous comparisons with cultivars carrying known alleles *(10)*.

The great number of gliadin components indicates that complex loci are responsible for synthesis of gliadins. In this investigation 27 alleles at the four *Gli* loci were determined (Table 1). The highest polymorphisms were found at the *Gli-A2* and *Gli-B2*. At the *Gli-A2* and *Gli*-B2 9 different alleles were determined, while 5 at the *Gli-A1* and 4 alleles at the *Gli*-B2 locus.

Frequency of the determined alleles was different and varied between 4.8% and 42.9%. The lowest frequency was found for *b* allele at the *Gli-A1* locus*, j, d, f* at the *Gli-A2, h, g, o, i,* at the *Gli-B2* locus. At each locus alleles with the highest frequency were established, and the highest frequency was found for *c* at the *Gli-A1* (33.3%), *a* at the *Gli-B1* (42.9%), and *a* at the *Gli-B2* (38.1%) Table 1. The high frequency of alleles could be connected (correlated) with the high adaptive value of some traits or related to the genes which control favor traits through selection *(6, 10)*.

The most frequent alleles at each *Gli-* loci were selected for analysis of their connection (correlation) with the contents of gluten. The majority of the analyzed cultivars (17 from 21) expressed good quality of gluten, the contents of which was higher than 27%. High frequent alleles were absent in cultivars (YG-2313, YG-5257, YG-3709, YG-6934) which had low gluten contents. Gliadin components encoded by *Gli-B1a* and *Gli-B1c* showed connection with high gluten contents. Earlier investigations by Pogna *et al. (15);* and Bechere *et al. (20)* showed that components 45-ã contributed to stronger gluten than components 42-ã. Numerous reports indicate that low-molecular weight glutenin subunits (LMW-GS), and high-molecular weight subunits (HMW-GS) together with gliadins, also influence the differences in quality traits of durum and bread wheat *(21, 22)*.



Electrophoregram of gliadins in cultivars *Triticum durum*:

3 (YG 3183)	7 (YG 5257)	6 (YG 5249)	13 (YG 4682)	17 (YG 7160)
4 (YG 4541)	8 (YG 5267)	6 (YG 5249)	14 (YG 5251)	18 (YG 7164)
5 (YG 5141)	9 (YG 5708)	11 (YG 6755)	15 (YG 6934)	↓ reference band of relative mobility 50
10 (YG 6281)	S (Bezostaya)	12 (YG 3709)	16 (YG 7154)	

Durum wheat contains a higher amount of protein contents than other cereal species. Apart from the quantity of protein, the quality of protein is very important. Many investigations have been focused on the nutritional value and protein composition of improvement of durum wheat. The role of gliadins in determination of dough extensibility and elasticity within the gluten matrix has been studied extensively. (Glutenins as well as the ratio of gliadins and glutenins are highly important for gluten quality *(12)*. It is well known that durum is used for bread products because of its high water binding capacity *(23)*.

Water absorption varied between 57.1% at cultivar YG-6934 and 58.8% at cultivar YG-7578 (Table 2). Both cultivars had the lowest quality number (0 and 34.5, respectively) and were classified in C_2 and C_1 quality class. For the remaining cultivars water absorption value ranged between 62.5% and 68.5%. Their quality number was higher than in the previous two cultivars. All of them be-

longed to the B_2 or B_1 quality class. Cultivars with quality number B_1 had quality number from 56.0 and 63.1, while cultivars that belonged to B_2 quality class had quality number from 45.1 to 52.8.

Analysis of association between alleles at a certain *Gli*-locus and gluten quality durum cultivars showed differences among them. A high positive association for gluten content was found for allele *c* and *h* at the *Gli-A1* locus, *c* and *a* at *Gli-B1*, than *c* and *b* at the *Gli-A2*, and *a* at the *Gli-B2* locus. Positive connection to water absorption was found for allele *c* and *a* at the *Gli-A1* locus, *c* and *a* at *Gli-B1*, followed by *c* and *b* at the *Gli-A2*, and *a* and *d* at the *Gli-B2* locus. The alleles *c* at the *Gli-A1* locus, *c* at *Gli-B1*, *b* at the *Gli-A2*, and *a* at the *Gli-B2* locus were the most frequent in cultivars which had the best value of gluten content and water absorption. This gliadin formula (*Gli*-A1*c*, *Gli*-B1*c*, *Gli*-A2*b*, *Gli*-B2*a*) could be used by breeders to make a concept of breeding to improve quality and gliadin composition.

CONCLUSIONS

Gliadins of 21 cultivars of *Triticum durum* were analyzed by the method of acid electrophoresis on polyacrylamide gel, and gliadin blocks identified. It was found that gliadin blocks differ according to the number, distribution and intensity of color of the components. On the basis of gliadin blocks 27 different alleles at 4 *Gli*-loci were determined. Polymorphysms of alleles also contributed to high genetic variability of durum species, in generally high genetic variability of plants. In this study the majority of analyzed durum cultivars showed good quality traits of gluten. Gliadin alleles at the *Gli-B1* locus showed strongest influence to gluten contents. High frequency of certain alleles could be related to some desirable agronomic traits which breeders favor during the selection process. It could be the basis for establishing correlation between the high frequency of alleles and analyzed traits of quality.

By pharinogram analysis of 21 durum wheat, the value of water absorption varied from 62.5% to 68.5% in 19 durum wheat. All of the 19 durum wheat had a higher quality number and belonged to B_1 or B_2 quality class. In that durum wheat the most frequent alleles occurred at the *Gli-A1* and *Gli-B1*. Correlation between gliadin alleles and analyzed traits of durum wheat indicate that gliadin alleles can be used as reliable genetic markers of agronomic and quality traits during the breeding process. The obtained results can be used in the process of improvement and selection of *Triticum durum*.

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