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SIMILARITY OF CULTIVARS OF WHEAT (*Triticum durum*) ON THE BASIS OF COMPOSITION OF GLIADIN ALLELES

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Twenty one durum wheat cultivars originating from different world countries were investigated. Composition of gliadins was analyzed by acid polyacrylamide gel electrophoresis. Allele composition of gliadins was determined on the basis of identified gliadin blocks. Polymorphisms of *Gli-* loci was established and 27 different gliadin alleles were identified, namely, 5 at *Gli-A1*, 4 at *Gli-B1*, 9 at *Gli-A2* and 9 alleles at *Gli-B2* locus. The catalogue of determined alleles was presented. Frequency of alleles ranged from 4.76% to 42.86%. Heterozygous *Gli-* loci were identified at two durum cultivars. Similarity among cultivars was studied on composition of *Gli*-alleles and presented by UPGMA dendogram. On the base of *Gli-* allele composition, similarity varied from 0% to 100%.

Key words: alleles, cultivar, durum wheat, electrophoresis, gliadin,

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INTRODUCTION

On the basis of biological function, proteins are classified into: metabolically active and storage proteins. Except that, proteins can be distinguished on the basis of wheat kernel: proteins of endosperm, proteins of aleuronic layer and proteins of germ. In most classifications we can differentiate four groups: albumins, globulins, gliadins and glutenins. Gliadins account for about 40-50% of the total proteins in wheat seeds and play an important role on the nutritional and processing quality of flour. Usually, gliadins could be divided into alpha (alpha/beta-), gamma- and omega- groups (QI *et al.,* 2006). 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 (GIANIBELLI *et al*., 2001). 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 (QIANG *et al*., 2004; MONDAL *et al.,* 2009). For each locus multiple allelisms were identified.

The polymorphism of *Gli* alleles plays an important role in increasing genetic variability (KNEŽEVIĆ *et al*., 2007, SHUAIB *et al.,* 2007). Due to considerable polymorphism of gliadins, electrophoresis is commonly used to identify wheat cultivars and lines. In any cultivar, one-dimensional electrophoresis may separate several dozens of unique gliadins (SHEVRY 2003; WAGA *et al.,* 2007). Electroforegrams obtained by this method are very suitable for allele identification on the basis of gliadin components which differ according to their number, mobility and colour intensity. Components are under the control of corresponding allele. By using this method many wheat cultivars have been identified (METAKOVSKY *et al.,* 1991; CLARK *et al*., 2001; NOVOSELSKAYA- DRAGOVICH *et al*., 2005; KNEZEVIC *et al.,* 2006). We have reported the results of our investigation on similarity of durum wheat cultivars according to their gliadin electrophoregrams. Thus, the objective of study was to investigate gliadin components composition in durum wheat and their identification on the base of gliadin polymorphisms.

MATERIALS AND METHODS

By electrophoretic analysis of gliadins, 21 cultivars of *Triticum durum* were examined. Extraction and separation of gliadins were carried out according to NOVOSELSKAYA *et al.* (1983), by polyacrylamid 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, which were diluted in 150 ml Al-lactate buffer (pH 3.1). Polymerisation of gel was initiated by 10 µl 3% hydrogen peroxide. Prepared solution was poured in vertically oriented apparatus, where between glasses plates gels were formed (dimension 150 x 150 x 1.8 mm). Sites for applying of samples were formed with 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 on the gel. Beside analyzed samples, extracts of gliadins of cultivars *Bezostaya*, *Langdon* and *Insignia* were placed as universal standards.

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

After performed electrophoresis, gels were immersed for 15 minutes in 300 ml of fixative, and after that stained in alcohol solution 0.05% Coomassie Briliant Blue R-250, where 250 ml 10% threechloroacetic acid was added. Next 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 base of comparison with electrophoregrams of standard cultivars (*Bezostaya, Langdon, Insignia*).

Mathematical and statistical data processing

Index similarity of composition of gliadin alelles of analyzed *Triticum durum* cultivars was computed by formula of SHEEN (1972).

Construction of dendograms was done on the basis of calculated similarity between all cultivars according to allele composition of *Gli* locus. Method of grouping (UPGMA = un-weighted pair group of mathematics average) of cultivars by numerical approximation was used. All analysis was carried out using a statistical package version 7.

RESULTS AND DISCUSSION

The analysis of electrophoregrams of cultivar *Triticum durum* shows that multipleallelism appears for each locus. Alleles differ regarding number, electrophoretic mobility and molecule mass of the components which are a part of the block content. 27 alleles separated into 4 series were put into the catalogue for 21 analysed cultivars, which corresponds to the number of gliadine-coding loci in *Triticum durum.*

On the basis of analysed cultivars of durum wheat, varying of allele composition of gliadine loci was determined (Table 1.)

In total, 27 alleles with four *Gli* loci were determined: 5 alleles with *Gli A1,* 4 alleles with *Gli B1,* 9 alleles with *Gli A2* and 9 alleles with *Gli B2.*

The composition of gliadin alleles was determined and it is characteristic for each cultivar (DJUKIĆ *et al.*, 2005; DJUKIĆ *et al.,* 2007). These alleles are responsible for the synthesis of a group of gliadin components which are called block. By identification of blocks of gliadin components on electrophoregram and their comparison with the catalogue of recognized gliadin blocks, alleles composition for each cultivar was determined (Table 1). Allele composition of gliadins indicates similarities and differences between cultivars (DJUKIĆ, 2004; DJUKIĆ *et al.,* 2008).

Table 1. Composition of gliadine alleles for analysed cultivars of Triticum durum

Cultivar ↓	*Gli - alleles*↓			
	A1	*B1*	*A2*	*B2*
1. YG 2313	*b*	*a*	*c*	*h*
2. YG 2591	*h*	*c*	*c*	*a*
3. YG 3183	*h*	*c*	*k*	*a*
4. YG 4541	*a*	*a*	*g*	*c*
5. YG 5141	*a*	*a*	*n*	*a*
6. YG 5249	*e*	*b*	*j*	*a*
7. YG 5257	*c*	*a*	*c*	*c*
8. YG 5267	*c*	*a*	*c + a*	*g*
9. YG 5708	*a*	*a*	*e*	*o*
10. YG 6281	*h*	*b*	*k + f*	*c*
11. YG 6755	*c*	*c*	*d*	*b*
12. YG 3709	*e*	*a*	*b*	*d*
13. YG 4682	*h*	*d*	*f*	*i*
14. YG 5251	*c*	*d*	*b*	*d*
15. YG 6934	*a*	*c*	*g*	*e*
16. YG 7154	*a*	*c*	*g*	*e*
17. YG 7160	*e*	*a*	*e*	*a*
18. YG 7164	*c*	*c*	*n*	*b*
19. YG 7578	*h*	*d*	*b*	*a*
20. YG 9052	*c*	*c*	*b*	*a*
21. YG 9674	*c*	*a*	*b*	*a*

The specific character of allele gliadins composition can be seen in the existence of the different allele from at least one locus (Table 1). However, for some cultivars, the same allele composition of gliadins was established. Only two cultivars have the identical gliatype (allele composition of *Gli* locus) – 15 (YG 6934) and 16 (YG 7154). Their gliatype indicates the following alleles: *a* from locus *Gli A1*; *c* from locus *Gli B1*; *g* from locus *Gli A2; e* from locus *Gli B2*.

The genetic formula of gliadin represents the cultivar identity. In most analyzed cultivars, the allele composition of gliadin shows homogeneity of gliadin loci. It means that one locus has one allele. However, some cultivars have two different alleles on one locus. Those are:

- cultivar 8 (YG 5267) alleles from *Gli A2* locus;
- cultivar 10 (YG 4541) alleles from *Gli A2* locus;

The existence of two alleles on one locus indicates the existence of heterogeneity of the cultivar for the given locus. The specified cultivars are heterogeneous on one separate locus.

By analyzing the composition of gliadin alleles for each cultivar of durum wheat, we determined the presence of some alleles from a particular *Gli* locus only in some cultivars. For example:
- *b* from *Gli A1*, only in cultivar 1 (YG 2313)
- *d* from *Gli A2*, only in cultivar 11 (YG 6755)
- *j* from *Gli A2*, only in cultivar 6 (YG 5249)
- *f* from *Gli A2*, only in cultivar 13 (YG 4682)
- *h* from *Gli B2*, only in cultivar 1 (YG 2313)
- *g* from *Gli B2*, only in cultivar 8 (YG 5267)
- *o* from *Gli B2*, only in cultivar 9 (YG 5708)
- *i* from *Gli B2*, only in cultivar 13 (YG 4682).

The specified cultivars of durum wheat 1 (YG 2313); 11 (YG 6755); 6 (YG 5249); 13 (YG 4682); 8 (YG 5267) i 9 (YG 5708) are characterized by the presence of alleles from particular gene loci, which were not found in other cultivars. The cultivar number 13 (YG 4682) and cultivar number 1 have two such alleles.

In the genetic formula of gliadin, in heterogeneous cultivars, out of two allele forms on one locus, the allele which has higher frequency for the given cultivar is the first, and the allele which is less prominent is the second. The first allele form was used for calculating the total frequency of this allele in analyzed cultivars (Table 2).

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

Gli A1		*Gli B1*		*Gli A2*		*Gli B2*	
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

By analyzing data from Table 2., it can be observed that the shown alleles had various frequencies in sorts *Triticum durum*. The frequency of alleles varied between 4,76%, determined for eight alleles (one from *Gli A1,* three from *GliA2* and four from *Gli B2* locus) and 42,86% calculated for allele from *Gli B1* locus.

For each *Gli* locus, two alleles with the highest frequency percentage were established:

- for *Gli A1* locus: allele *c* (33,33%) and alleles *h* and *a* (with 23,8% each);
- for *Gli B1* locus: allele *a* (42,86%) and allele *c* (33,33%);
- for *Gli A2* locus: allele *b* (23,8) and allele *c* (19,05);
- for *Gli B2* locus: *a* (38,09) and allele *c* (14,28).

The exception is locus *Gli A1,* where we separated three alleles because of the equal value of the percentage of frequency of allele *h* and allele *a*.

The alleles from *Gli B* locus have high frequency, from *Gli B1,* 42,86% (*a*) and 33,33% (*c*) and from *Gli B2* 38,09% (*a*).

The largest number of allele forms (9), was discovered in *Gli A2* and *Gli B2* locus.

Out of 21 analyzed cultivars of *Triticum durum*, heterozygosity was discovered in two cultivars, which is 9,52%. The analysis of alleles composition (Table 1.) shows that the prominent heterogeneity on loci of both cultivars exists on *Gli A2* locus. Theoretically speaking, polymorphism of all *Gli* loci is possible. Therefore, it can be said that 1,00 (4/4 = 1,00) loci is polymorphous. However, in our analyses only one locus was polymorphous in heterogeneous cultivars. Given in proportion, we can say that regarding those cultivars, 0,25 (1/4=0,25) loci is polymorphous. In addition to expressed differences in alleles frequency, on most gene loci there are different alleles or alleles groups.

Allele *h* is located only on *Gli A1*and in five different cultivars *Triticum durum* (2 - YG 2591, 3 - YG 3183, 10 - YG 6281, 13 - YG 4682, 19 - YG 7578).

Allele *n* is located on *Gli A2* locus in two different cultivars (5 YG 5141 and 18 YG 7164).

Four alleles (*j, f, o, i*) are located only on one gene locus of particular cultivars of *Triticum durum.* For example: in cultivar 6 (YG 5249) allele *j* from *Gli* locus *A2* is seen; allele *f* is seen only in cultivar 13 (YG 4682), and exists only on *Gli* locus *A2*; allele *o* is seen in cultivar 9 (YG 5708), and exists only in *Gli B2* and *i* allele is seen only in cultivar 13 (YG 4682), and exists in *Gli B2*.

Electrophoregrams of gliadins were used for determining the alleles of gliadin loci. Allele composition, which was characteristic for each sort, was used for calculating similarity coefficient. On the basis of obtained data, dendograms of analyzed cultivars were made.

The values of similarity coefficient for compared cultivars ranged from 0-100%, in dependence on how many same alleles on gliadin loci two compared cultivars had. All analyzed cultivars were compared one with the other.

The obtained dendogram with Euclidean distances depicts mutual similarities and differences of gliadin alleles of analyzed cultivars of durum wheat (figure 1). Cultivars have the same ordinal number as in previous analyzes. Smaller groups (clusters) of mutually similar cultivars can be observed.

Cultivars 7 and 8 have the first pair of genetically similar gliadin alleles 75%. Cultivar 1 joins the cultivars 7 and 8 and they form a cluster, because the

similarity coefficient of cultivar 1 and cultivar 7 is 50%, as well as similarity coefficient of cultivar 1 and cultivar 8 (50%).

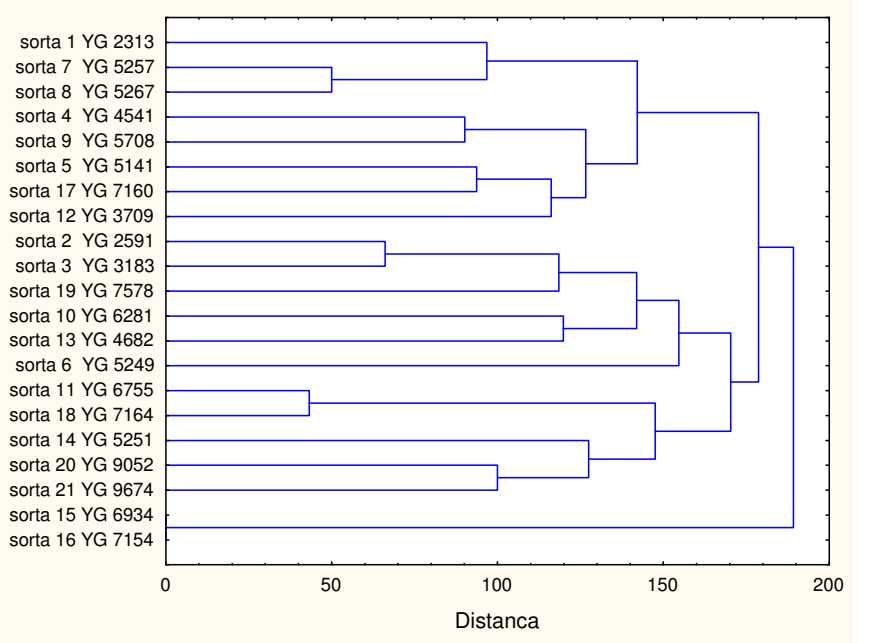

Figure 1. UPGMA dendogram of *Triticum durum* cultivars obtained on the base of coefficient of gliadin alleles similarity

The second group (cluster) is made of cultivars 4, 5, 9, and cultivar 17 and cultivar 12 are connected to them. The similarity coefficient of gliadin alleles between cultivars 4 and 5, 4 and 9 and 5 and 9 is 50%. Cultivar 17 joins them; it is less similar to cultivar 4 (25%), but the similarity coefficient with cultivars 5 and 9 is 50%. Cultivar 12 also belongs to this cluster; it has only 1 same allele (25%) with cultivars 4, 5 and 9, but the genetic similarity of gliadin alleles with cultivar 17 is 50%.

The third cluster consists of cultivars 2, 3, 10, 13 and 19. The second and third cultivars make a pair, because their mutual similarity is 75%. Cultivar 19 joins them, because the similarity coefficient of cultivar 19 and 2, and 19 and 3 is 50%. This cluster also contains cultivars 13 and 10, which also make a pair, and are connected to the cultivars from this cluster by similarity coefficients with cultivars 13 and 19 - 50% and cultivars 10 and 3 - 50%. Even though it is more different than similar, cultivar 6 also belongs to this cluster. Cultivar 6 is absolutely different from

cultivar 10, coefficient of similarity of gliadin alleles is 0%, but the similarity coefficient with all other cultivars from this cluster (2, 3, 13 and 19) is 25%.

The fourth group consists of cultivars 20, 21, 14, 11 and 18. Cultivars 20 and 21 make a pair, because their mutual similarity is 75%. Cultivar 14 joins them; it is 50% genetically similar to 20th and 21st cultivar. The pair of cultivars 11and 18 also belong to this cluster; their coefficient of similarity of gliadin alleles is 75%, and one other cultivar has genetic similarity of 50% with cultivar 20.

In the end, we have a pair of genetically identical cultivars 15 and 16, because the coefficient of similarity of gliadin alleles is 100%.

CONCLUSION

In this paper the gliadins of 21 cultivars of *Triticum durum* were analyzed by the method of electrophoresis on polyacrylamide gel and gliadin blocks were identified.

- It was discovered that gliadin blocks differ in number, layout and intensity of coloration of components.
- On the basis of analyzed electrophoregrams, varying of allele composition of gliadine loci was established. 27 alleles with 4 gliadin loci were determined in total. The highest polymorphism is shown by *Gli A2* and *Gli B2*
- It was determined that the following cultivars of durum wheat: 1 (YG 2313); 11 (YG 6755); 6 (YG 5249); 13 (YG 4682); 8 (YG 5267) and 9 (YG 5708), are characterized by the presence of alleles from particular gene loci, which were not found in other cultivars. Cultivar number 13 and cultivar number 1 have two such alleles.
- It was discovered that alleles frequency varies from 4,76% (for 8 alleles) to 42,86% for allele *a* from *Gli B1*.
- Out of 21 analyzed cultivars of *Triticum durum*, in cultivar 2 (8 YG 5267 and 10 YG 6281) heterozygosity was discovered, which is 9,52%. Shown heterozygosity per loci in both cultivars is on *Gli A2*.

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SLIČNOST SORTI PŠENICE (*Triticum durum*) NA OSNOVU KOMPOZICIJE GLIJADINSKIH ALELA

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I z v o d

Proučavana je 21 sorta pšenice *Triticum durum* poreklom iz različitih zemalja sveta. Metodom acid poliakrilamidne gel elektroforeze, analizirana je kompozicija glijadina. Urađena je identifikacija glijadinskih alela na osnovu analize identifikovanih glijadinskih blokova komponenti na elektroforegramima. Ustanovljena je polimorfnost *Gli-* lokusa i identifikovano 27 različitih *Gli-* alela i to: 5 alela na *Gli-A1*, 4 na *Gli-B1*, 9 na *Gli-A2* i 9 na *Gli-B2*. Urađen je katalog determinisanih alela. Učestalost determinisanih alela je varirala od 4.76% do 42.86%. Heterozigotnost *Gli*-lokusa je nađena kod dve sorte. Na osnovu kompozicije glijadinskih alela analizirana je sličnost između ispitivanih sorti i predstavljena na dendogramu. Na osnovu kompozicije glijadinskih alela, sličnost između sorti se kretala od 0% do 100%.

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