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BIOHEMICAL ANALYSIS OF GLIADINS OF WHEAT *Triticum ðurum*

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ABSTRACT. Gliadins are one of the major protein fractions, which are deposited in endosperm protein bodies of grain. Composition of gliadin components in 21 durum wheat cultivars was separated by acid polyacrylamide gel electrophoresis. The obtained electrophoregrams by poliacrilamid gel electrophoresis were used for analysis of similarity of investigated durum wheat cultivars. Relative mobility and coloring intensity of components of gliadins (prolamins) were estimated and used for compilation of electrophoretic formulas for all cultivars. Electrophoretic formula for each cultivar was specific. Number of components per cultivar varied between 18 and 28. Variability of presence of some components indicates high polymorphysms of gliadins. Similarity among cultivars according to relative mobility of gliadins was from 9.52% to 61.53%.

INTRODUCTION

Quality of wheat can assume according to protein quality and quantity of grain. On the base of biological function, proteins are classified on: metabolicaly active and storage proteins. Except that, proteins can be distiguish on the base of morphology of wheat kernel: proteins of endosperm, proteins of aleuronic layer and proteins of germ. The mostly acceptable classification is Osborn's clasification on the base of protein solubility. By this classification we can differentiate four groups: albumins, globulins, gliadins and glutenins.

Great numbers of biochemical, genetical and technological investigations of wheat proteins have been conducted including electrophoretic characterization (Jones et al., 1983; Autran et a1., 1987; Kudryavtsev et a1., 1994; Knezevic and Lookhart, 1996; Fernandez et a1., 2002; Peyron et a1., 2002). Electrophoregrams of proteins provide information about identity of cultivars, protein polymorphisms, technological quality of grain, flour and dough of wheat (Mecham et al., 1978; Draper, 1987; Menkovska et al., 2002, Djukic, 2004). Polyacrylamide gel electrophoresis has been developed for identification of wheat cultivars by 'fingerprinting' their gliadin proteins (Bushuk and Zillman, 1978; Lookhart et al., 1982). Results of intensive investigation of wheat proteins during last decades reflect in fact that they are better studied than proteins of other cereals. By using of new techniques for separation and isolation shows complexity of these proteins. For example, according to Lasztity (1996) only gliadin´s fraction of endosperm proteins of single wheat variety can be separated on 46 components, using gel electrophoresis. Many researchers have pointed out the relationship between technological qualities and the composition of gliadin components (Sozinov and Poperelya, 1984; Autran, 1987; Chakraborty and Khan, 1988; Knezevic et al., 1994; Lookhart

et al., 2001). Great complexity of storage wheat proteins sugests that accession to problems only on the base of solubility is not enough for understanding, on molecular level, different protein fractions, according to technological, agronomical and biological properties generally. Because of that, in studing of relation between gliadins and biological properties it is necessary to make start from gliadin spectrum.

The reproducibility of analysis and the high resolution of gliadin components separation by electrophoresis on polyacrylamide gel are perform electrophoresis as one of the best methods for separation and visualization of proteins (Romac et al., 1999).

We have reported the results of our investigation on similarity of durum wheat cultivars according to their gliadin electrophoregrams. Thus, the objective of this study was to investigate gliadin components composition in durum wheat and their identification on the base of gliadin polymorphisms.

MATERIAL AND METHODS

Grain samples of 21 cultivars of Triticum durum were supplied by Institute for Small Grains Kragujevac. Gliadins were extracted by ethanol and separation of gliadins was carried out according to method of Novoselskaya et al., (1983), by poliacrilamid gel electrophoresis at pH 3.1. It was used 8.33% polyacrylamide gel, prepared with: 12.5 g acrilamid, 0.62 g N,N'-methylenebisacrylamide, 0.15 g ascorbin acid, 200 µl 10% ferosulfate heptahydrate, which were diluted in 150 ml Al-lactate buffer (pH 3.1). Polymerisation of gel was initiated by 10 µl 3% hydrogen peroxid. Prepared solution was poured in vertically oriented apparatus, where between glasses plates were formed gels (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% ethil alcohol, according to procedure that was described in paper of Knezevic (1992). From each cultivar 20 µl of extract was applied on the gel by micropipette. On one gel 17 samples were analized. Beside analized samples, it was placed extract of gliadins of cultivars Bezostaja, Langdon and Insignia, as universal standards.

Separation of the gliadin molecules was performed during 2.5 to 3 hours, in electrical circuit under constant voltage from 550 V and in 5-mM aluminum lactate buffer. At the begining of analisys, temperature of electrophoretic buffer was 10°C, while at the end was 25-30°C.

After performed electrophoresis, gels were immersed 15 minutes in 300 ml of fixative, and after that stained in alcoholic solution 0.05% Coomassie Briliant Blue R-250, where was added 250 ml 10% threechloroacetic acid. Staining was carried out during night. Next day, solution of stain was poured off. Gels were washed in water and photographed. Photographs are used for determination of bands and their relative mobility according to method of Bushuk and Zillman (1978).

Index similarity of pairs of gliadin components of the same relative mobility of analyzed *Triticum durum* cultivars was computed by formula of Sheen (1972):

$$S = \frac{\text{pairs of similar match bands}}{\text{pairs of similar match bands + different match bands}}$$

Construction of dendograms was done on the base of counted similarity of pairs of gliadin components of the same relative mobility among all cultivars. It was used method of

grouping (UPGMA = unweighted pair group of mathematics average) of cultivars by numerical approximation (Ferguson, 1980).

RESULTS AND DISCUSSION

By electroforetic analysis of gliadins 21 cultivars of *Triticum durum* were comprised. On the base of obtained electrophoregrams their identification was done. Gliadin elecrophoregrams represent "finger prints" of wheat cultivars (Konarev et al., 1979; Lookhart et al., 1982; Draper, 1987). In the table 1. relative mobility of gliadin components and evaluation of their coloring intensity are presented.

Obtained electrophoregrams are different for analyzed cultivars of durum wheat in respect of presence of some components, relative mobility and coloring intensity (the darkest colored band marked by 5, and the lightest colored as 1). Electrophoregram of one cultivar was compared with electrophoregrams of the rest 20 cultivars. It was found for one cultivar characteristic presence in range from 18 to 28 gliadin components. Similar data about number of bands (from 22 to 30 per cultivar) separated by one-dimensional electrophoresis reported by Knezevic et al., (1990); Metakovsky et al., (1987) found about 30 gliadin components characteristic for cultivar separated on electrophoregram; Wrigley et al., 1982 (ratio was from 15 to 30 of gliadin components for one cultivar). However, separation of gliadins on 20 to 50 components is possible by electrophoresis (Wrigley and Shepherd 1973; Mecham et al., 1978; Brown and Flawell 1981). Great number of gliadin components indicates that complex loci are responsible for synthesis of gliadins.

With respect that the analyzed cultivars of *Triticum durum* were identified on the base of composition of gliadin components, we used electrophoretic formula of gliadins of investigated cultivars for comparison of the numbers of pairs gliadin components of the same relative mobility for compared cultivars. We compared all analyzed cultivars for each other. Values of similarity coefficient for compared cultivars were from 9.25 to 61.53%. On the base of obtained values (coefficient of relative mobility) dendogram of analyzed cultivars was done (fig. 1). Method of grouping (UPGMA) of cultivars on the base of numerical approximation was used.

Table 1. Electrophoretic formulas of gliadins for analyzed of durum wheat cultivars

r.m.	10									20									30									40									50									60									70									80							
Sort	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8
01. YG-2313										1	3			3		1			4	4						4			4				3		4			2			3		3					2				2		2			1		1																
02. YG-2591										1	1			3		3		1			4	4						4		4				3		4		3				4	3			2	1		2			3				3			2		1														
03. YG-3138					1	1					1					3		2		2			3		3	3									2		2				1		2		2			2		2			2	2							2			3		2													
04. YG-4541										1			2			2				3		3				1					3			2			3			1		2			2				1		1					2		1			1		1														
05. YG-5141						1				1			2			2				3		3		3		2		4		4			3				3		3			3			2				2			3				3		2			1		1														
06. YG-5249							1			1			1		3		2		2	3		4	3		1			1		4				2			3		2		3		3		2		1			1		2				3		2	1			1			1			1										
07. YG-5257						1				1		3			3				2		3	2							2		3	3		2			3			3				4		3			2	1			3				3		2			1										1						
08. YG-5267						1				1		2			3		3			4			3	3		2		3		3			3			3			2		2		2			2				3			3				3			1			1		1												
09. YG-5709								1		1		1			2			2		2			2		2		3									4	4		5			3				5				1		4			4		3		2	2									1		3						
10. YG-6281										1		1			1				1	2	2		1		1		2		3		3		2				2				3					3			2				2				1			1		1		1			1											
11. YG-6755						1			1		1				1	1		2		2	3		3				2		2		3		3			2			2		2		2			2			2				3			2			2			1				1												
12. YG-3709				1				1				3			4		5			4		4		4		4		3			4				3			3		3	3					3			3		3				3				3		2		1				1											
13. YG-4682									1			2			3		4			3		3		3		3	2	2			3			4		3			1			4			3			3					2				3			3					2		1			1		1									
14. YG-5251												1			2		2		1					2		2			1			2							3			4			3			1				2				1		2			2					2		2	2			1									
15. YG-6934						1				1		2			3		4			3		3		3	3		3	3			4				4			2		3			3		2		2		3				3		3		3		3			3			3			2				2									
16. YG-7154						1		1				2			2					2		3					2		3		4		4			2			3		3			3			3			3			2		2		2		3					21			2		2		1										
17. YG-7160				1		1				1		2			3		3			2		3			3				2		3				4				3		2			3			3		2			2				2			2				2			2		2	2									
18. YG-7164				1		1				1		1		2		3	4			4	3		3		3	3			3		3				4		4			4			3		1		1				3			4			3				3			3		2		1		2									
19. YG-7578				1		1					2		3			3		4		3		3			3		4		3		4				4	3		3		3		4	3				3			2		2		3			3						3	2															
20. YG-9052				1		1					1		1		3			3				3	3		4		3		3				3				3			3		3		3		3		3				3			3		3		2			2	2															
21. YG-9674						1	1			1			2			3			2			4			1		2						3				4				3				3		3			2			2			3		3		2		2		1		1														
Sort	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8
r.m.	10									20									30									40									50									60									70									80							

Relative band intensity: 1 is lightest, 5 is darkest. All gliadin bands were distributed between 14 and 82 units.

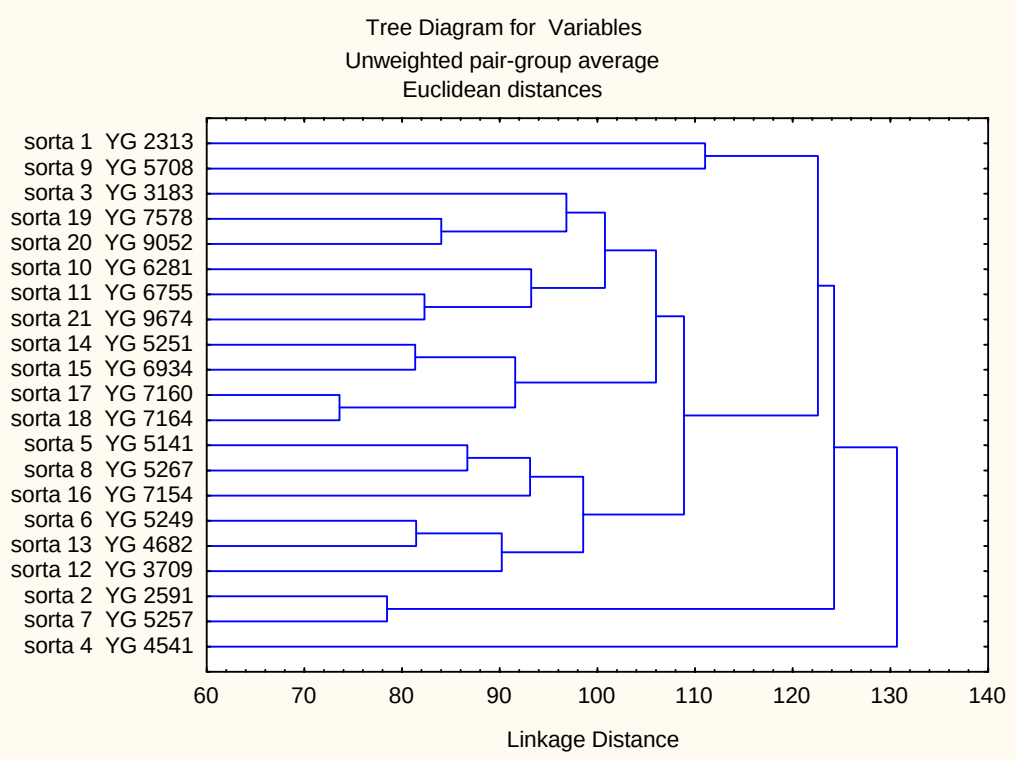

Figure 1. UPGMA dendogram of *Triticum durum* cultivars obtained on the base of comparison of number of pair of the gliadin components with same relative mobility for compared cultivars

Obtained dendogram with distance according to Euclid (Euclidean distances) indicates on mutual similarities and differences on the base of number of pairs of gliadin components of the same relative mobility for two compared cultivars. Observing dendogram it can be easy find pairs and smaller groups (clusters) of mutually similar cultivars.

The first pair in dendogram makes cultivars 1 and 9, whose coefficient of similarity of relative mobility is 32.5%. This pair of cultivars is very different from all other cultivars. The same case is also with cultivars 2 and 7, which show great mutual similarity of pairs of gliadin components of same relative mobility (60.46%). At the same time, we observed great difference of this pair from other cultivars. Besides them in dendogram is single cultivar 4, which is the most different in relation to all other.

Cultivars 19 and 20 are mutually similar in 53.06%. Cultivar 3 join to them and it is similar to cultivar 19 in 46.8%. These three cultivars make cluster with cultivars 10, 11 and 21. Similarity of pairs of gliadin components of the same relative mobility of cultivars 11 and 21 is 50%. Cultivar 10 joins to them and it is similar with cultivar 21 in 50%.

The greatest similarity of pairs of gliadin components of the same relative mobility shows cultivars 17 and 81 (61.53%). This pair of cultivars make cluster (group) with another pair of analyzed cultivars, 14 and 15, whose coefficient of gliadins components of the same relative mobility is 54.16%. Two pairs of cultivars form cluster because the coefficient of similarity of cultivars from the pairs are mutually great: cultivars 15 and 18 - 50.9%; cultivars 15 and 17 - 50.98%; cultivars 14 and 18 - 48.97%. This group with two pairs mutually similar cultivars is connected with previous cluster.

Next cluster form pairs of cultivars 5 and 8 (coefficient of similarity 54.54%), to whom cultivar 16 and pair of cultivars 6 and 13 (52.17%) join, and to whom cultivar 12 join,

because it is similar with cultivar 13 in 45.83%, and with cultivar 6 coefficient of similarity of pairs of gliadin components of the same relative mobility is 46.51%.

CONCLUSIONS

Polyacrylamide gel electrophoresis represents efficient method for gliadin analysis that we used in this investigation of 21 cultivars of *Triticum durum*. Electrophoregrams of durum wheat gliadins obtained by acid PAG electrophoresis were used for measuring of band motilities and visually estimation of bands color intensities. Each cultivar had specific electrophoregram. Analyzed durum wheat cultivars were different in relation to their composition of gliadin components. Number of registered components varied between 18 and 28 per cultivars. Different number of components and their relative mobilities and color intensities can use for estimation of cultivar differences. The high polylimorphisms of gliadins was established that could be explaining by different origin and pedigree of cultivars. Also, possible changes by recombination and mutation of gliadin controlling genes can contribute to increasing of gliadin polymorphisms.

Electroforegrams, also, can also use for study of cultivar similarity toward to gliadin compositin. In this study, similarity of cultivars established on the base of coefficient of similarity and five clusters of similar cultivars has been obtained. By comparison of cultivars, according to pairs of components of the same relative mobility we came to cognition that some cultivars, for example, cultivar 17 (YG 7160) and cultivar 18 (YG 7164) are very similar (61.53%), but also there in analyses, cultivars that are very different in relation remain analyzed cultivars, for example cultivar 4 (YG 4541). The obtained results can be used in program of breeding improvement and selection of *Triticum durum*.

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