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BIOCHEMICAL ANALYSIS OF 21 CULTIVARS *Triticum durum*

Nevena Đukić[1], Desimir Knežević[2], Slavica Solujić-Sukdolak[1]

[1]*Faculty of Science, University of Kragujevac,*
R. Domanovića 12, 34000 Kragujevac, Republic of Serbia
e-mail: nevena@kg.ac.yu

[2]*Faculty of Agriculture Zubin Potok, University of Priština, Republic of Serbia*

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ABSTRACT: In this work, the variability of amino acid composition of 21 cultivars of *Triticum durum* is analyzed by using method of one-dimensional and two-dimensional chromatography in different systems of organic diluents. Using biochemical analysis of amino acids in biological material of *Triticum durum*, we got: 21 chromatograms (diluent: n-butanol: acetic acid: distilled water); 8 chromatograms (of chosen cultivars) in diluent: phenol: distilled water, and a chromatogram of two-dimensional separation of amino acids in cultivar 20 (YG 9052) according to which amino acids were qualitatively determined. The most present is glutamine amino acid, proline, valine and dioxy-phenylalanine. Glutamine acid was identified in twelve chromatograms of analyzed cultivars of *Triticum durum*. The presence of glutamine acid is very important regarding the fact that it is of unique value for metabolism of nitrogen in cell, as well as for the primary synthesis of proteins.

Key words: wheat, proteins, nutritive value, amino acids

INTRODUCTION

Wheat is the most important bread cereal: due to its spread, production, consumption and nutrition. Regarding its flavour, nutritional and biological value, wheat products or flour products are high above the other cereals. (LJUBISAVLJEVIĆ, 1999) Almost 70% of people diet is based on various products of grain origin. Large part of other products comes from animals that eat grains. Therefore, there is an extensive investigation of biochemical and nutritive contents of grain, and the largest part refers to the grains of bred cultivars. A chemical content of grain is, in the first place, determined by genetic factors, environmental conditions in the period of grain maturation, as well as by human factors - selection (NEŠKOVIĆ *et al.*, 2003; KNEŽEVIĆ *et al.*, 2007a).

According to the structure compactness, wheat is divided into hard and soft. Hard wheat *Triticum durum* has higher percentage of proteins and gluten, so protein quality is

higher as well. It is known that protein quality in grain represents an important factor to the quality of wheat (ĐUKIĆ *et al.*, 2006a; ĐUKIĆ *et al.*, 2007; KNEŽEVIĆ *et al.*, 2007b). KHAN *et al.*, (2000) pointed out that, few years ago, Quality Trait Locus (QTL) for high protein proportion on short branch of 6b chromosome of *Triticum turgidum* was found (using marker FA 15-3). In their work, they presented new molecular markers that should facilitate GPC-gene transfer (gene responsible for high protein proportion in tetraploid and hexaploid wheat culture).

Cereal wheat component is very important qualitative feature of *durum* wheat (CHEE *et al.*, 2001) Wheat contains a blend of 20 amino acids, which determine characteristic features of proteins. Amino acids have their particularities regarding metabolic transformation and participation in biosynthesis of organic constituents (ĐUKIĆ et al. 2006b)

The purpose of this investigation was analysis of variability of amino acid composition in 21 *Triticum durum* wheat cultivars.

MATERIALS AND METHODS

For the purposes of the work, grain specimens of durum cultivars from different parts of the world, were used. The cultivars are listed in Table 1.

Table1. Analyzed cultivars of *Triticum durum*

1. YG 2313	*Var. Mamovdi From Algeria*
2. YG 2591	*Var. Senatore Capeeci From Italy*
3. YG 3183	*Yuma From USA*
4. YG 4541	*Var. Bidi 17 From Australia*
5. YG 5141	*Var. Tehvacun c-6 From Mexico*
6. YG 5249	*Var. Leeds From USA*
7. YG 5257	*Var. Grane From Mexico*
8. YG 5267	*Var. Sarpatti Gamoran From India*
9. YG 5708	*Var. Hercules From Canada*
10. YG 6281	*Var. Patrizin From Italy*
11. YG 6755	*Var. Trimagria From Italy*
12. YG 3709	*Var. C.1.12.928 From Columbia*
13. YG 4682	*Var. Stemart 63 From Canada*
14. YG 5251	*Var. S-9 From India*
15. YG 6934	*Var. Rolette From USA*
16. YG 7154	*Var. Creso From Italy*
17. YG 7160	*Var. Cocorit ' s ' From Cimyt*
18. YG 7164	*Var. Kuperaunda From Cyprus*
19. YG 7578	*Var. Durtal From France*
20. YG 9052	*Var. Cando D-7057 From USA*
21. YG 9674	*Var. Regal From France*

Certain amounts of free amino acids can usually be found in biological material (apart from the other substances such as carbohydrates, lipids, inorganic salts etc.) The extraction of amino acids from complex compound of grain can be conducted by using 80% of ethanol and sedimentation of dissolved proteins by chloroform (GRUJIĆ - INJAC, 1962). For identification and determination of amino acids from a compound, most suitable method is chromatography because it is simple, selective and can be used as micro analytical method as well as for identification and determination of ingredients of very complex compounds (TRAJKOVIĆ et al., 1983). One-dimensional chromatography showed

that large number of amino acids was found in amino acid extract so we conducted their extraction by using two-dimensional chromatography (DŽAMIĆ, 1989).

For developing of chromatograms, different diluents systems are used: solution of n-butanol and fed up water solution of phenol, reagent used for coloring stains is 0.2 % solution of ninhydrin in 99% acetone. All amino acids apart form the secondary, give blue-purple colored product reacting with ninhydrin in conditions mentioned. Tone and intensity of color depends from kind and concentration of amino acids. Proline and hydroxyproline together with ninhydrin give yellow colored compound, which is transformed into red compound with surplus ninhydrin on high temperature.

Identification of some amino acids is conducted by using comparison with test substances (GRUJIĆ - INJAC, 1962)

RESULTS AND DISCUSSION

Results of qualitative analysis of free amino acids in investigated durum cultivars were achieved based on 21 chromatograms (developed in diluent: n-butanol: acetic acid: distilled water), Table 2. Chromatograms produced in phenol diluent: distilled water (Table 3) and also based on two-dimensional chromatography (Fig. 1). Rf values in amino acids of these chromatograms were compared to standard Rf values for given diluents.

Qualitative analysis showed that the most present amino acids are: glutamine acid, proline (together with oxyproline), dioxyphenilalanine, valine and threonine. The presence of essential amino acids is very important because it surely influences nutritive value of durum wheat (ĐUKIĆ, 2004). In different cultivars of investigated durum wheat, 6 essential amino acids were found: valine, threonine, phenylalanine, tryptophan and arginine. The most present essential amino acid is valine. Very much present essential amino acid in investigated durum wheat cultivars is threonine (chromatograms: 7, 9, 10, 12, 15, 16, 18, 19, 20 and 21). Threonine is essential amino acid which is included into different protein groups contents. In an organism, it can be used for biosynthesis of pirol nucleus and other compounds important for metabolism of cell. With the activity of threonine aldolase enzyme, threonine can be transformed into glycine and acetaldehyde.

Glycine was identified in chromatograms of the cultivars under following numbers: 1, 3, 4, 5, 10, 11 and 14. It is the simplest monoaminomonocarbon acid but it is a very important starting substance for biosynthesis of a number of organic constituents such as: proteins, creatine, glutation, serin-glycoaldechyde, glioxil acid, purines, porfirines and other compounds. Glycine can be synthesized, apart from threonine, from glioxil acids, serine and sarcosine. Sarcosine (present in chromatograms 1, 3, 4, 5, 10, 11, 13, 14 and 15) chemically represents methyl-glycine. Glycine and phormaldechyde are produced form of sarcosine under influence of sarcosin oxydase.

Presence of glutamic acid is the most important because it is of great importance for nitrogen metabolism in cell, especially in primary synthesis of amino acids (KNEŽEVIĆ et al., 2007; LASZTITY, 1984). High proportion of glutamic acid and proline is pointed out by CHARBONNIER *et al.* (1980). Comparing to the other amino acids, glutamic acid is most present in chromatograms of investigated cultivars (1, 3, 4, 5, 6, 8, 10, 11, 14, 15, 16 and 20). In gliadins, almost entire contents of glutamic acid are present in the form of glutamine (LASZTITY, 1996). Glutamines are source of nitrogen that can be used as a food for wheat germination and emergence. From biochemical point of view, nitrogen storage in form of glutamine is the most economic way of using it (KNEŽEVIĆ, 1992). Glutamic acid and glutamine are non-essential amino acids and they belong to the group of glycogenic amino acids. Products of glutamine acid decomposition serve for biosynthesis

of sugar where its glycogen character comes from. Glutamic acid is synthesized due to Krebs cycles. In the process of synthesis, ammoniac (produced by reduction of nitrites) is introduced in α–ketoglutar acid. Glutamic acid serves as a precursor for proline formation. Together with proline and arginine, glutamic acid takes part in alkaloids biosynthesis.

Apart from participating in purine formation, glutamine also serves as a donor – NH_2- group for biosynthesis of amino sugars. Glutamine can be produced by introducing NH_3 in glutamic acid with one mole of ATP. In the presence of ATP, glutamic acid turns into γ-glutamyl phosphate that in the presence of ammoniac and glutamyl-sintetase enzyme, gives glutamine and one mole non-organic phosphate in the form of H_3PO_4 γ-glutamyl phosphate can be transformed into proline using range of mid-products and the most important is glutamic acid semialdehyde. It is a very unstable compound which undergoes the process of dehydration and losing one molecule of water turns into proline-5-carbon acid that in the presence of proper reduktase gives proline. Proline is important stress factors redactor for plants (NEŠKOVIĆ *et al.*, 2003). Proline was identified in 9 of 21 chromatograms of investigated *durum* wheat cultivars (6, 8, 9, 15, 17, 18, 19, 20 and 21). Oxyproline is produced by oxidizing of proline, which was previously activated. This was confirmed by the results of investigating proline and oxyproline that were both marked with N15. It was also asserted that carbon chain of both acids can be used for biosynthesis of glucose, which means that both amino acids are glucogene. In this process, proline is first oxidized into glutamic acid in the presence of enzymes, and then glutamic acid turns into α-ketoglutar acid which becomes glucose. It is known that oxyproline is present in cell walls and plant membranes or glume that envelopes the seed.

Qualitative analysis in diluent phenol: distilled water (Table 3) of amino acids present in chosen chromatograms: 2, 6, 8, 10, 13, 16, 19 and 20; enabled identifying some free amino acids that did not appear in the previous diluent. For instance, on chromatogram 2, cultivar YG 2592, in diluent n-butanol: acetic acid: distilled water, valine was identified and in diluent phenol: distilled water, on chromatogram 2, agrinin-chlorid, sarkosine, norleucine and α-amino carbon acid were identified.

On chromatogram 6, we identified alanine which couldn't be determined in diluent n-butanol: acetic acid: distilled water, because of the same standard Rf value glutamic acid. Analysis in diluent phenol: distilled water (although on a smaller number of investigated cultivars) shows higher proportion of phenylalanine (chromatograms of cultivars under numbers 6, 8, 10, 13 and 16). Phenylalanine is essential amino acid which means it is a necessary component of diet. In organism, phenylalanine is metabolized in different ways. Most of it (about ¾ of phenylalanine brought in with diet) is decomposed by process of hydroxylation by which is transformed into tyrosine, and then further on through catabolism of tyrosine. Minor part of phenylalanine is metabolized with transamination and decarboxylation. A part of phenylalanine is incorporated into proteins. Phenylalanine (same as tryptophan and tyrosine) belong to aromatic amino acids. Their common feature is formation of aromatic rings in a molecule from pure alphatic origin substances. Mechanism of aromatization functions through shikimic acid. Next key intermediate is chorismic acid which can form: anthranilic acid - serves as a precursor for synthesis of tryptophan, and prephenic acid - forms phenylalanine and tyrosine. Biosynthesis of aromatic amino acids is mutually regulated.

Tryptophan was identified on chromatograms of cultivars under numbers: 3, 4, 5 8, 9, 12, 20 and 21). Presence of tryptophan in higher plants is significant because it serves as source for formation IAA (indol-3-acetic acid), also known as auksyne (stimulator of plant growing). In fact, all three aromatic amino acids are precursors of many secondary metabolic products. Apart form the hormones with indolil ring, tryptophan forms indolil phitoalexines and glucosinolates. Phenylalanine and tyrosine form hydroxycinnamon acid,

many phenol compounds, phlavonoids and isophlavonoids, alkaloids and lignin. Methionine was identified in many of the investigated cultivars (chromatograms 3 and 9). Regarding to the fact that methionine, together with cystine and cystenine, belongs to group of amino acids which contain S, its identification has its significance. Presence of S or disulphide is important for the secondary and tertiary structure of molecules. Intramolecular disulphide links are present in globular structure of gliadins. Methionine is also important as a precursor of plant hormone etilen and prolamine (NEŠKOVIĆ *et al.*, 2003).

Regarding the presence of most amino acids in investigated material, their extraction on chromatogram 20, was conducted by using two-dimensional chromatography (Fig. 1). According to comparisons of Rf values of butanol and phenol solution, with standard Rf values, amino acids: proline and valine are present on chromatogram 20; with minor exceptions to standard Rf values- tryptophan according to comparison of Rf values with standard Rf values in diluent phenol: distilled water, arginin-chloride was identified; and according to match with standard Rf values in diluent n-butanol: acetic acid: distilled water, threonine and dioxy-phenylalanine were recognized.

CONCLUSIONS

Using the biochemical analysis of prolamine (gliadin) of *Triticum durum* wheat, the following conclusions were made:

Glutamic acid, proline, dioxy-phenylalanine, valine, and threonine were the most present among free amino acids in biological material of *Triticum durum* wheat cultivars.

Using qualitative analysis of chromatograms, six essential amino acids were identified: valine, threonine, phenylalanine, leucine, tryptophan and arginine. The most present among them was amino acid valine. Presence of essential amino acids surely has great influence to nutritive value of durum wheat.

Table 2. Qualitative analysis of amino acids on 21 chromatograms
(diluent: n- butanol: acetic acid: distilled water)

amino acids ↓ cultivars of durum wheat→	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
sarcosine	+		+	+	+					+	+		+	+	+						
proline						+		+	+						+		+	+	+	+	+
oxyproline																	+				
glycine	+		+	+	+					+	+			+							
threonine							+		+	+		+			+	+		+	+	+	+
α-aminokapronine							+	+	+							+					+
glutamic acid	+		+	+	+	+		+		+	+			+	+	+					+
valine		+	+		+				+	+	+	+				+	+	+		+	
norvaline	+			+		+		+						+	+				+		
dioxyphenylalanine	+		+		+		+			+		+	+	+		+			+	+	+
phenylalanine									+						+						
norleucine								+								+				+	
metionin			+						+												
tryptophan			+	+	+			+	+			+							+	+	
arginine					+	+			+		+	+				+		+		+	+

Table 3. Chromatograms (of chosen cultivars) in diluent: phenol: distilled water

amino acid ↓ / chosen hromatograms→	2	6	8	10	13	16	19	20
arginine	+	+	+	+	+	+	+	+
valine								+
alanine		+						
dioxy-phenylalanine				+				+
sarkosine	+		+		+	+		
norvaline							+	
proline			+					+
leucine	+							
phenylalanine		+	+	+	+	+		
tryptophan						+	+	+
α-amino kapronine acid.	+	+	+	+			+	

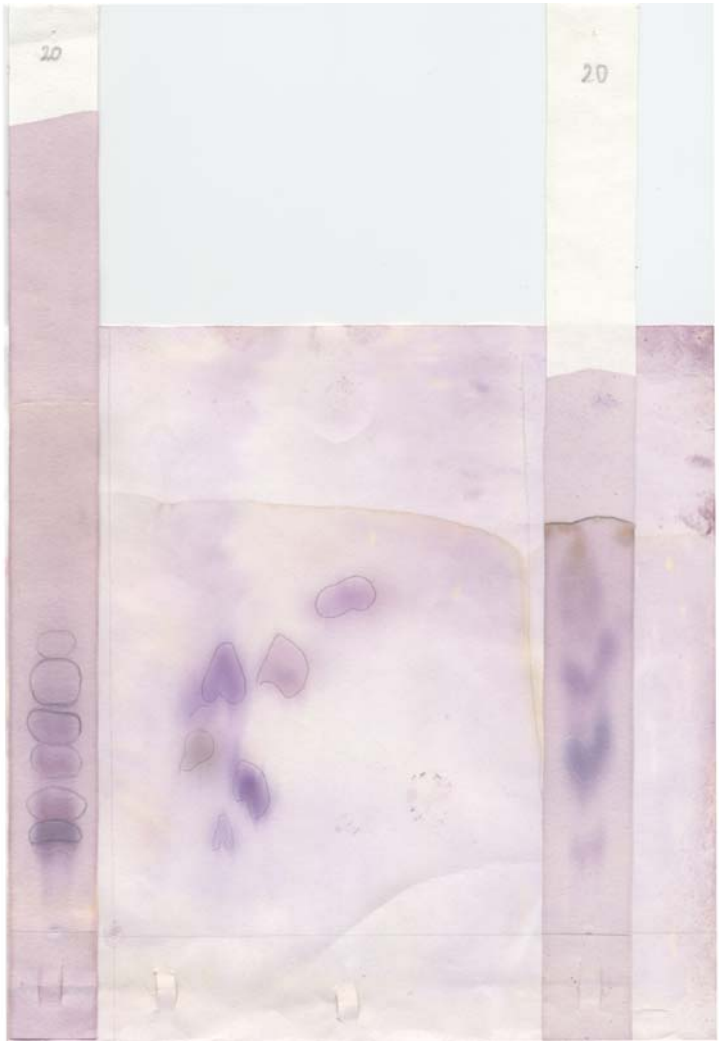

Figure 1. Chromatogram of two-dimensional separation of amino acids in cultivar 20 (YG 9052)

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