STUDY ON THE FREE AMINO ACID PROFILE OF LEAVES FOR GRAPES VARIETIES OF GALBENĂ DE ODOBEŞTI SORTOGROUP

Marioara BOSOI[1], Constantin ŢÂRDEA[2]

[1]Research and Development Station for Viticulture and Oenology Odobeşti, Stefan cel Mare no. 61, 625300, Odobeşti, Romania
[2]University of Agricultural Sciences and Veterinary Medicine Iaşi, Mihail Sadoveanu Avenue no.3, Iaşi, Romania

Corresponding author email: mioara_bosoi@yahoo.com

Abstract

Researches on amino acid profile carried out so far in the genus Vitis showed its great variability, suggesting importance of the genetic involvement in this variability This paper presents preliminary results of investigating the amino acid profile from leaves to the grape varieties of sortogroup Galbenă de Odobeşti. The grape varieties taken in the study were: Galbenă de Odobeşti, Bătută neagră, Zghihară de Huşi, Negru moale and Negru vârtos. For extraction of free amino acids from the leaves of the vine has been used the method Bieleski & Turner (1966) adjusted for grapevine, and separation technique was performed by thin-layer chromatography (TLC). Identification and quantitative estimation of free amino acids was performed using analysis and image processing software - ImageJ ver.1.46. The amount of free amino acids ranged between 18.76 mg / mg fresh leaf at Galbenă de Odobeşti variety and 14.33 mg / mg for Negru moale variety. In the varieties Galbenă de Odobeşti, Bătută neagră and Zghihară de Huşi the most abundant free amino acids were: aspartic acid (Asp), glutamic acid (Glu), serine (Ser), threonine (Thr), proline (Pro) and glycine (Gly). The predominant amino acids for Negru moale variety were: aspartic acid (Asp), serine (Ser), glycine (Gly), glutamic acid (Glu), threonine (Thr), proline (Pro) and arginine (Arg). Amino acids predominantly to Negru vârtos variety are: aspartic acid (Asp), glutamic acid (Glu), serine (Ser), threonine (Thr), proline (Pro) and arginine (Arg). Aspartic acid (Asp) is found in large quantities compared with other amino acids, representing 31.7% of total amino acids identified for Negru vârtos variety, 31.5% at Zghihară de Huşi variety, 30.5% fpr Negru moale variety, 27. 5% for Galbenă de Odobeşti variety and 25.3% for the variety Bătută neagră. Statistical ratio Pro / Arg and amino predominant ratio (Asp / Ser), genetically differentiate the Bătută neagră variety the other varieties belonging to the sortogroup Galbenă de Odobeşti, with a confidence interval of 95%. These preliminary data provide a basis for further research that can demonstrate that amino acid profile of the leaves can be used as method in discriminant analysis of grape varieties.

Key words: amino acid profile, Galbenă de Odobeşti, sortogroup, thin-layer chromatography.

INTRODUCTION

The fund management in grape germplasm is one of the most important issues is the growing concern of researchers from all countries. Classical identification methods based on characteristics ampelographic / botanical not the most accurate, with certain restrictions due to instability morphological characteristics influence environmental conditions.

Research on amino acid profile made so far in the genus *Vitis* showed its great variability, suggesting importance of the genetic involvement in this variability (Kliewer et al., 1966, Kliewer 1969; Klub et al., 1978; Marcy et al. 1981; Notsuka et al., 1984, Huang and Ough, 1991). On vines, the total amino acids increases during aging reaching values between 200 and 6500 mg/l of each amino acid variations from one year to another, and from one variety to another (Ţârdea, 2007). Share amino acids in grapes is high and is 20 -30% of the total nitrogen compounds (Poux and Ournac, 1970). Total amino acid content especially predominant ratio of amino acids differ significantly from one variety to another, so knowing the amino acid spectrum of vegetative organs and grapes can be a means of differentiation of vine varieties in terms of genetically (Hernández-Orte et al., 1990). Studies on free amino acid profiles of grapes made so far reported variations in the genetic material and suggests new biochemical descriptors (Shiraishi, 1996).

The characterization of the grapevine varieties based on free amino acid profile shows a great importance to complement the modern methods on investigation of the vine varieties (isoenzymatic analysis and DNA analysis).

MATERIALS AND METHODS

The biological material was represented by five local varieties to Galbenă de Odobeşti sortogroup (Galbenă de Odobeşti, Zghihară de Huşi, Bătută neagră, Negru moale and Negru vârtos), belonging ampelographic collection of Research and Development Station for Viticulture and Winemaking Odobeşti. Samples for analyses were the young leaf obtained by forcing cuttings - eye. The leaf samples (2 or 3 leaves, ~ 5 g) were collected from each variety in plastic bags, labeled, stored on ice and brought to laboratory for analyses.

For extraction of free amino acids from the leaves of the vine has been used the method Bieleski & Turner (1966) adjusted for grapevine. This method ensures the extraction, separation and quantitative estimation of amino acids from small samples of vegetal fresh tissue, by thin layer chromatography technique. For extraction of free amino acids in biological material and for the preparation of standard solutions of amino acids was used a mixture solution with methanol, chloroform and water in a ratio of 12:5:3 v/v/v. For each grape variety was weighed a sample of 0.5 g biological material. The fresh leaves were lyophilized and crushed in a mortar with liquid nitrogen until was obtained a fine powder. 0,200 g was transferred for each sample in a Eppendorf tube 1.5 ml. Over powder was added 200 ml extraction solution (methanol: chloroform: water - M/C/W) in ratio of 12/5/3 (v/v/v), which allowed the removal of pigments and lipids from plant material in chloroform layer. Cell suspensions were treated with sonic frequencies for 1 hour at 4 ° C, causing rupture cell membranes. After sonication the samples were stored in a refrigerator at 4 º C for at least 1 h. To obtain the supernatant containing the amino acids, the sample was centrifuged at 13,500 rpm for 15 minutes at 4 ° C.

The chromatographic separation of amino acids was achieved on TLC plates with silica gel stationary phase 60 on aluminum foil 20 x 20 (TLC Silicagel 60), manufactured by Merck KGaA, Germany. TLC plates were made with size 10 x 7 cm and have been marked the areas of application for samples. Using standard amino acids were produced by Merck KGaA, Germany, in the solution of methanol: chloroform: water (12:5:3 v / v / v). Samples (2 μl) were applied with an automatic micropipette with volume control, from left to right at the bottom of the plate, in the areas marked for each cultivar. The chromatographic separation or development of plates was done in mono dimension, was used the development system based on n - butanol / glacial acetic acid / water (ratio 4:1:1 v/v/v). After chromatographic separation, the TLC plates are ready for viewing and identification of amino acids separated. To ensure reproducible results, the tests were repeated three times for predominant amino acids, to all five varieties.

View amino acids was performed using specific reagents − ninhydrin (a solution in concentration of 0,25%). Amino acids appear as separate spots colors. The TLC plates viewed ninhydrin solution, sensitive to light were scanned or photographed for the qualitative and quantitative evaluation of free amino acids separated from each sample. To identify amino acids separated in samples was calculated the retention factor (Rf) compared with each standard amino acid. Quantitative determination of free amino acids in the samples was performed using image analysis program - ImageJ ver 1.46 r, and the values obtained were expressed in μg free amino acid for 1 mg fresh leaf tissue.

The data obtained were processed statistically for analysis of variance (ANOVA test) and has been determined significance of differences for the total free amino acids content and the ratio between the predominant amino acids (Student test). Analysis of variance and Student test was performed using the Microsoft Excel menu Tools - Data Analysis.

RESULTS AND DISCUSSIONS

14 amino acids were identified. In Figure 1 and 2 are presented TLC plates with amino acids separated and visualized with ninyidrin solution for the five cultivars studied. Standard amino acids used: alanine (Ala) and histidine (His). In

Figure 3 is presented the amino acid profile in leaves (chromatogram) to the cultivars of Galbenă de Odobeşti sortogroup, with standard glutamic acid (Glu). The amino acid profile for the variety Galbenă de Odobeşti is presented in figure 4.
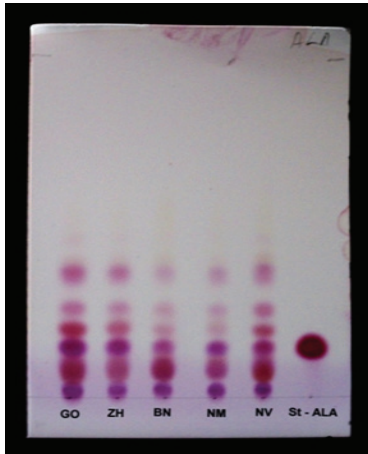

Figure 1. TLC plate with amino acids separated and visualized (St.– Ala)
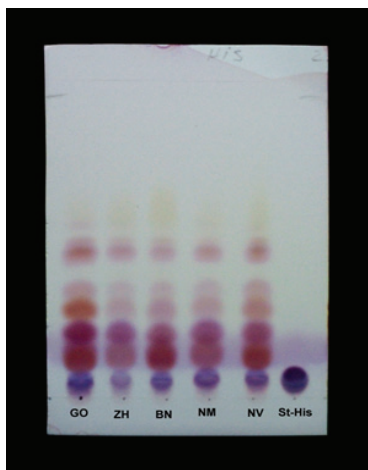

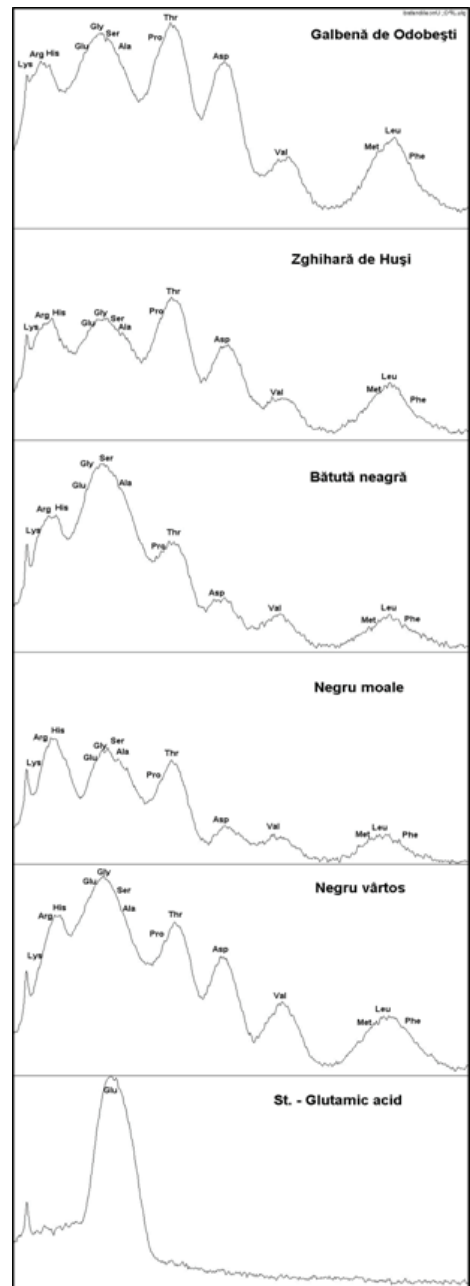Figure 2. TLC plate with amino acids separated and visualized (St.– His)

Figure 3. Amino acid profile of leaves for the varieties analyzed (St. - Glu)
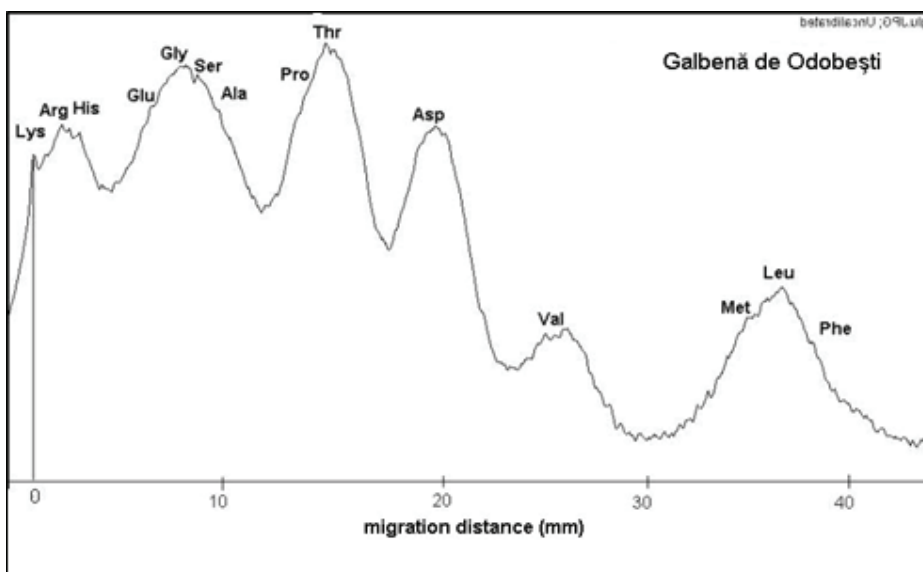
165

Figure 4. Amino acid profile of the leaves to cultivars Galbenă de Odobeşti

The results with the free amino acids content in leaves to the cultivars of Galbenă de Odobeşti sortogroup, expressed in µg/mg fresh leaf tissue and percentage are summarized in Table 1. The total content of free amino acids in leaves of vine varieties of Galbenă de Odobeşti sortogroup varied between 18.76 µg/mg fresh leaf tissue in the variety Galbenă de Odobeşti and 14.33 µg/mg fresh leaf tissue to the cultivar Negru moale, with intermediate values for the cultivars Zghihară de Huşi (14.54 µg/mg), (cv)

Bătută neagră (15.20 µg/ mg) and (cv) Negru vârtos (17.74 µg/ mg). Aspartic acid (Asp) is found in much higher amounts than other amino acids identified, representing 31.7% of total amino acids identified to the cultivar Negru vârtos, 31.5% to the variety Zghihară de Huşi, 30.5% to (cv) Negru moale, 27.5% to (cv) Galbenă de Odobeşti and 25.3% to the variety Bătută neagră.

Table 1. Free amino acid content in the leaves at vine varieties of Galbenă de Odobeşti sorto group

The variety / Amino acid	Galbenă de Odobeşti µg/mg	%	Zghihară de Huşi µg/mg	%	Batută neagră µg/mg	%	Negru moale µg/mg	%	Negru vârtos µg/mg	%
Aspartic acid (Asp)	5.15	27.5	4.58	31.5	3.84	25.3	4.37	30.5	5.62	31.7
Glutamic acid (Glu)	2.04	10.9	1.37	9.4	2.17	14.3	1.30	9.1	2.06	11.6
Serine (Ser)	2.21	11.8	1.73	11.9	1.82	12.0	1.65	11.5	2.06	11.6
Threonine (Thr)	2.04	10.9	1.75	12.0	1.59	10.5	1.13	7.9	1.83	10.3
Proline (Pro)	1.28	6.8	0.82	5.6	1.46	9.6	1.13	7.9	1.15	6.5
Arginine (Arg)	0.99	5.3	0.71	4.9	0.87	5.7	0.88	6.1	1.26	7.1
Glycine (Gly)	1.27	6.8	0.86	5.9	0.91	6.0	1.35	9.4	0.71	4.0
Lysine (Lys)	0.93	5.0	0.72	5.0	0.81	5.3	0.76	5.3	0.81	4.6
Alanine (Ala)	1.09	5.8	0.75	5.2	0.56	3.7	0.53	3.7	0.72	4.1
Leucine (Leu)	0.81	4.3	0.69	4.7	0.49	3.2	0.45	3.1	0.50	2.8
Histidine (His)	0.65	3.5	0.37	2.5	0.47	3.1	0.54	3.8	0.54	3.0
Phenylalanine (Phe)	0.11	0.6	0.05	0.3	0.04	0.3	0.06	0.4	0.15	0.8
Valine (Val)	0.13	0.7	0.07	0.5	0.09	0.6	0.11	0.8	0.20	1.1
Methionine (Met)	0.06	0.3	0.07	0.5	0.08	0.5	0.07	0.5	0.13	0.7
Amino acid content	18.76	100.0	14.54	100.0	15.20	100.0	14.33	100.0	17.74	100.0

The most abundant of the free amino acids in leaves were:
- to the variety Galbenă de Odobeşti: aspartic acid (Asp), serine (Ser), threonine (Thr), glutamic acid (Glu), proline (Pro) and glycine (Gly), representing 74.7% of total amino acids identified;
- to the variety Zghihară de Huşi: aspartic acid (Asp), threonine (Thr), serine (Ser) and glutamic acid (Glu), representing 64.8% of total amino acids content;
- to the variety Bătută neagră: aspartic acid (Asp), glutamic acid (Glu), serine (Ser), threonine (Thr), and proline (Pro), representing 71.7% of the amino acids identified;
- to the variety Negru moale: aspartic acid (Asp), glycine (Gly), serine (Ser), glutamic acid (Glu), threonine (Thr), and proline (Pro), representing 76.3% of total amino acids content;
- to the variety Negru vârtos: aspartic acid (Asp), glutamic acid (Glu), serine (Ser), threonine (Thr), arginine (Arg) and proline (Pro), represents 78.8% of total amino acids content;

Statistical interpretation of data obtained on total amino acid content in leaves of vine varieties belonging to Galbenă de Odobeşti sortogroup by analysis of variance/ANOVA test shows that there is no statistical significance between the five varieties, because the value of P is 0.938854, greater than the critical value of P, 0.05.

The ratio proline/arginine and the ratio of predominant amino acids (aspartic acid/serine) are considered parameters what remain constant from year to year and differ significantly from one variety/cultivar to another and can be called descriptive biochemical parameters. The ratio Pro/Arg has values between 0.91 to the cultivar Negru vârtos and 1.68 for the cultivar Bătută neagră and the ratio Asp / Ser ranges from 2.11 to the variety Bătută neagră and 2.73 to the variety Negru vârtos (Table 2).

Statistical interpretation of experimental data obtained for the ratio Pro/Arg by analysis of variance/ANOVA test shows the existence of a statistical significance between the five cultivars because P value is less than the critical value of P, that is 0.05 (Table 3).

Table 2. The values of biochemical descriptors for grapevine varieties to Galbenă de Odobeşti sortogroup

The variety/ Biochemical descriptors	Galbenă de Odobeşti	Zghihară de Huşi	Bătută neagră	Negru moale	Negru vârtos
Pro/Arg ratio	1,29	1,15	1,68	1,28	0,91
Asp/Ser ratio	2,33	2,65	2,11	2,65	2,73

Table 3. The significance of differences to the ratio proline / arginine in the leaves of vine varieties to Galbenă de Odobeşti sortogroup

The vine variety	Galbenă de Odobeşti	Zghihară de Huşi	Bătută neagră	Negru moale	Negru vârtos
Galbenă de Odobeşti	0				
Zghihară de Huşi	0.06542	0			
Bătută neagră	0.00051	0.00064	0		
Negru moale	0.94536	0.06777	0.00093	0	
Negru vârtos	0.01346	0.06722	0.00096	0.01368	0

Statistical the ratio proline/arginine genetically differentiates the variety Bătută neagră the other varieties of Galbenă de Odobeşti sortogroup, with a confidence interval of 95%. A significant difference exists between the variety Negru vârtos and the varieties Galbenă de Odobeşti and Negru moale. Variance

analisys/ANOVA test for the ratio predominant amino acids (aspartic acid/serine), statistically differentiates the variety Bătută neagră by the other varieties except the variety Galbenă de Odobeşti with a confidence interval of 95%, while this difference is not significant for the variety Galbenă de Odobeşti (Table 4).

Table 4. The significance of differences in the ratio aspartic acid / serine of leaf vine varieties of Galbenă de Odobeşti sortogroup

The vine variety	Galbenă de Odobeşti	Zghihară de Huşi	Bătută neagră	Negru moale	Negru vârtos
Galbenă de Odobeşti	0				
Zghihară de Huşi	0.11292	0			
Bătută neagră	0.23710	0.00732	0		
Negru moale	0.08869	0.93531	0.00357	0	
Negru vârtos	0.06510	0.44839	0.00510	0.39683	0

CONCLUSIONS

The total free amino acids content in leaves varies between 18.76 μg/mg fresh leaf tissue to the variety Galbenă de Odobeşti and 14.33 μg/mg to the variety Negru moale, with intermediate values for the variety Negru vârtos (17.74 μg/mg), (cv) Bătută neagră (15.20 μg/mg) and (cv) Zghihară de Huşi (14.54 μg/mg).

The most abundant of the free amino acids in leaves of native varieties to Galbenă de Odobeşti sortogroup are: Asp. Ser. Thr. Glu. Pro. Arg and Gly. Aspartic acid is found in large amounts compared with other amino acids predominant.

The ratio proline/arginine has differentiated statistically the variety Bătută neagră by the other varieties of Galbenă de Odobeşti sortogroup with a confidence interval of 95%.

Significant difference exists between the varieties Galbenă de Odobeşti and Negru vârtos and between the varieties Negru moale and Negru vârtos.

The ratio between predominant amino acids (aspartic acid/serine) has differentiated genetically the variety Bătută neagră by the other varieties (except the variety Galbenă de Odobeşti) with a confidence interval of 95%.

There is significant difference between the varieties Galbenă de Odobeşti and Negru vârtos and between the varieties Negru moale and Negru vârtos.

The amino acid profile of leaves can be used as discriminant analysis method of vine varieties to Galbenă de Odobeşti sortogroup. with the other modern methods of investigation (isoenzyme analysis and DNA analysis).

REFERENCES

Bieleski R.L.. Turner N.A., 1966. Separation and estimation of amino acids in crude plant extracts by thin-layer electrophoresis and chromatography. Anal. Biochem., p. 278–293.

Hernández-Orte P., Guitart Ana, Cacho J., 1999. Changes in the Concentration of Amino Acids During the Ripening of Vitis vinifera Tempranillo Variety from the Denomination d'Origine Somontano (Spain). Am. J. Enol. Vitic. 50:2, p. 144-154.

Huang Z., Ough C. S., 1991. Amino Acid Profiles of Commercial Grape Juices and Wines. Am. J. Enol. Vitic. 42:3, p. 261-267.

Kliewer W.M., 1967. Annual cyclic changes in the concentration of free aminoacids in grapevine. Am. J. Enol. Vitic. 18., p. 126–137.

Shiraischi Mikio, 1996. Proposed Biochemical Descriptors for Amino Acids to Evaluate Grape Germoplasm, J. Japan. Soc. Hort. Sci. 65(2), p. 283-289.

Ţârdea C., 2007. Chimia si analiza vinului, Ed. "Ion Ionescu de la Brad, p. 754-770.