A STUDY ON THE USE OF THE AMINO ACID PROFILE AS A BIOCHEMICAL DESCRIPTOR FOR THE CHARACTERIZATION OF THE VINE GERMPLASM

STUDIU PRIVIND UTILIZAREA PROFILULUI DE AMINOACIZI CA DESCRIPTOR BIOCHIMIC PENTRU CARACTERIZAREA GERMOPLASMEI VITICOLE

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Abstract. *Due to a large number of traditional cultivars, new varieties and clones, it is imperatively necessary to identify resources of vine germplasm with the purpose of further manipulation. In this respect, researches on the analysis of free amino acids in various Vitis cultivars, conducted during different stages of growth and development, have highlighted great variability in the total amino acid content and their profile. Although the amino acid content differs each year, the ratio of certain amino acids (Pro, Arg, Glu, Gln, Tre, Ser, Ala) remains constant in each cultivar and may be used as in indicator in the genetic differentiation between cultivars of the same area. This paper aims at evaluating, based on research conducted worldwide so far, the posibilities of devising new methods, techniques and working schemes which may lead to the precise genetic identification of cultivars.*

Key words: vine germoplasm, amino acid profile, genetic identification

Rezumat. *Datorita existentei unui numar mare de cultivare traditionale, soiuri si clone noi este imperativ necesara identificarea in vederea manipularii resurselor de germoplasma viticola. In acest sens, cercetari privind analiza aminoacizilor liberi la diferite cultivare apartinand genului Vitis, efectuate in diferite stadii de crestere si dezvoltare, au evidentiat o variabilitate mare sub aspectul continutului total de aminoacizi si profilului acestora. Desi continutul in aminoacizi difera de la un an la altul, raportul dintre anumiti aminoacizii (Pro, Arg, Acidul glutamic, Glu, Tre, Ser, Ala) ramane constant la fiecare cultivar si poate fi utilizat ca indice de diferentirere genetica intre cultivarele din acelasi areal. Lucrarea de fata isi propune, pe baza cercetarilor efectuate in lume pana la aceasta data, o evaluare a posibilitatilor in vederea formularii unor metode, tehnici si scheme de lucru care sa conduca la identificarea genetica de precizie a cultivarelor.*

Cuvinte cheie: germoplasma viticola, aminoacid, identificare genetica

INTRODUCTION

The evaluation of the genetic diversity in *Vitis vinifera* L. is important not only in studies about different varieties and cultivars but also in studies concerning the improvement and preservation of the germplasm fund of the species. The classic identifying methods, based on botanical and ampelographic characteristics are not entirely the most precise, with certain limitations due to the instability of morphological characteristics under the influence of environmental

factors. Where improvement is concerned, the identification of the varieties in the first stages of development is extremely difficult, since the particular characteristics appear gradually over the next few years. Also, in the case of rootstocks the ampelographic methods of identification can only be used in rootstock plantations, but not after grafting.

An important step in evaluating the genetic material has been taken with the introduction of biochemical methods at protein level through izoenzymatic analysis, which reveals differences between the accumulation of proteins or enzymes coded by various alleles (Schwennesen et al., 1982; Crespan et al., 1999), as well as mollecular techniques based on DNA which have been in use for over two decades and which have the ability to identify extremely fine variations or polymorphism given by DNA sequences (Striem et al., 1990;Bourquin et al., 1991). Also researches concerning amino acid profiles in the *Vitis* genus have shown great variability (Kliewer et al. 1966, Kliewer 1969, 1970, Kluba et al., 1978, Marcy et al., 1981, Notsuka et al., 1984, Huang et Ough, 1991), pointing toward the great importance of implicating genetics in this variability. This study aims at evaluating researches in this area in order to devise a methodology for the identification of cultivars in this manner.

MATERIAL AND METHOD

Examining the amino acid profile has been an issue in various research papers for viticulture and oenology. (Peynaud et Maurie, 1953,; Peynaud et Lafon-Lafourcade, 1961;Ough et Amerine, 1966,; Ough,1968, Poux et Ournac, 1970, Gallander, 1974, Kluba et al.,1978, Amerine et al.,1979).

Amino acids are monomeric forms of proteins, synthesized only by plants, with an important role in the growth and development process. There are more than 500 amino acids in nature, however only 24 are proteinogenic in the genetic code, present in all living organisms, 20 were identified in *Vitis Vinifera* L. as well, in various stages of growth and development. The main amino acids present in must at harvest and their quantities are shown in Table 1.

Table 1

The main amino acids present in must at harvest (Țârdea C., 2007)

The name of the amino acid	The quantity in mg/l of must		
	Minimum	Maximum	Medium
proline	40	3800	750
arginine	55	1200	350
glutamic acid	53	270	140
threonine	9	130	85
serine	5	81	36
asparatic acid	15	100	35
triptophane	5	31	31
alanine	7	260	30
lysine	5	63	28
glycine	2	42	22
leucine	3	58	18
tyrosine	2	75	15

fenilalanine	4	62	15
histidine	8	26	12
valine	ND	11	11
isoleucine	2	10	3
metionine	ND	15	-
hydroxyproline	ND	14	-
ornitine	ND	5	-
cysteine	ND	2	-
Total of aminoacids, mg/l	215	6255	1581

According to the data presented by the author in the paper "Wine Chemistry and Analysis" (Ţârdea C., 2007), the total amino acid content in must vary significantly, between 200 and 6500 mg/l. The total amino acid amount increaseses over the ripening process, with variations in each amino acid from one year to another. During ripening, only proline increases significantly, ten to fifty times, while other amino acids increase two or three times. (Lafon-Lafourcade et Guimberteau, 1962, Kliewer, 1968, Kluba and al., 1978, Shiraishi and al.,1986).

RESULTS AND DISCUSSIONS

Preliminary research on free amino acids in the *Vitis* genus have been conducted in 1966 by P. W. Mark Kliewer, A. R. Nassar, and H. Olmo, through examining leaves and berries from 23 species of the *Vitis* genus during 3 development stages. 32 amino acids have been identified in different species. High contents of hydroxyproline and other unidentified amino acids have been present in leaves and unripened berries (samples taken in June and July) in 5 species**:** *V. aestivalis, V. berlandieri, V. cinerea, V. rufotomentosa,* and *V. simpsoni 'pixiala'*. There have been significant differences in quantity, in various *Vitis* species, concerning the concentration of amino acids such as arginine, proline and asparagine.

Changes in free amino acid concentrations have been noticed in three important varieties of *Vitis labrusca* Bailey (Concord, Catawba, and Delaware) during ripening (Richard M. Kluba , Leonard R. Mattick , and L. Ross Hackler, 1978). The most representative free amino acids in the three varieties have been alanine and arginine. At ripening, their concentration varied from 29,4 to 97,8 mg/100 ml must for alanine and 32,8 to 48,0 mg/100 ml must for arginine, which translates into 30,8 %, and respectively 22,2% of the total of free amino acids. Other amino acids have values ranging between 1 and 10 mg/100 ml must. The reason for the presence of alanine in high quantities in the *V. labrusca* Bailey varieties, compared to the *V. vinifera* L. has not been determined so far.

Other studies concerning establishing the free amino acid concentration in must conducted during 1986 -1990 (Sara E. Spayd si Joy Andersen-Bagge, 1996) in 12 *Vitis vinifera* L. varieties cultivated in Columbia and Yakima Valley, have shown that arginine (measured in mg/l) has been the predominant amino acid in the Gewurtztraminer, Muscat Canelii, Semillion, and Pinot noir varieties, with significant variations each year, while proline has been predominant in the

Chenin blanc, Chardonnay, Cabernet Sauvignon, Grenache, Limberger, Merlot, Sauvignon blanc, and White Riesling varieties.

In the case of table grape varieties there have also been variations in the amino acid content and their profile. For example, in the Cardinal and Moldova varieties, though with relatively close total amounts of free amino acids (340 mg/l, and respectively 363 mg/l), there have been differences in their profile. Arginine was the predominant amino acid in the Moldova variety while the Cardinal variety had accumulated important quantities of glycine, tyrosine, valine, histidine, isoleucine (Abramov,S.A.;Vlasova,O.K.; Daudova, T. I.,2001).

A number of 18 amino acids has been determined from the baginning of the ripening process until harvest, over 3 years in the Tempranillo, Riesling italian, Cabernet Sauvignon, and Moristel varities in the Spanish region of Somontano (Puri Hernández-Orte, Ana Guitart, si Juan Cacho, 1999). Arginine, proline, histidine and glutamine have been the highlighted amino acids in all 4 varieties. The 4 predominant amino acids have amounted to 62% to 88% from the total of amino acids. During the 3 years, in the Riesling and Cabernet Sauvignon varieties, the predominant amino acid had been proline and in the Tempranillo variety, arginine was predominant. However, in the Moristel variety arginine had been predominant for a year and proline for 2 years.

Due to the ratio of the 4 amino acids (proline, arginine, glutamine and histidine), as well as the sum of all amino acids showing significant differences from one variety to the other, a conclusion has been reached: it can be used as an indicator for differentiating varieties of the same area..

Relatively recent researches on determining the profile of free amino acids in the ripened berries have been conducted in various vineyards from Australia in six *Vitis vinifera* L. varieties (Cabernet Sauvignon, Grenache, Muscat Gordon, Pinot Noir, Riesling and Sangiovese), cultivated in the same conditions and harvested in the same stage of ripening (A.P. Stines, J. Grubb, H. Gockowiak, P.A. Henschke, P.B. Høj si R. van Heeswijck, 2000). In this case there have been differences in content, however proline and arginine have been the predominant amino acids in all six varieties, represented between 65% and 82% from the total of amino acids. In the Cabernet Sauvignon varieties the berries have shown a high content of proline but a decreased content of arginine, while the other varieties had medium content of both proline and arginine.

In all cases the proline accumulation happened mostly at the end of the ripening period, approximately 4 weeks after the process had begun. The accumulation of arginine had started before ripening and continued until full ripening, except for the varieties which accumulated large quantities of proline, in which case arginine concentration reached a certain level earlier in the growth and development process. These observations concerning the metabolism of the two amino acids have led to the hypothesis of possible interrelations.

Since the amino acid profile in must is usually composed of more than 10 amino acids, there is no validity in the comparison between cultivars based on

the individual amino acid levels. In this respect, Huang si Ough (1991) have demonstrated through highlighting the genetic differences betwen six varieties of *Vitis vinifera* L., that the Pro/Arg ratio is characteristic to each cultivar, concluding after the statistic analysis that the Pro/Arg ratio may be used as a descriptor of various cultivars.

Studies conducted in Japan (Mikio Shiraisch, 1996) on a number of 259 vine germplasm varieties including varieties, hybrids, rootstocks, wild vines, have shown that the amino acid content of the *Vinifera* varieties and hybrids has significantly surpassed that in rootstocks and the wild species observed. Concerning amino acids, arginine (Arg), alanine (Ala) and glutamic acid (Glu) have been predominant compared to asparatic acid(Asp), threonine (Tre), serine (Ser), valine (Val), methionine (Met), isoleucine (Ile) and leucine (Leu). Based on the content of fundamental amino acids, the vine genetic material has been classified into the following types or profiles: Glu, Arg, Ala, Arg +Ala and Ala + Arg. Moreover, for an easy assessment of amino acid composition a new biochemical descriptor has been proposed, the γ ratio:

γ = TRE+SER+ALA/([ASP+GLU]+[VAL+MET+ILE+LEU+ARG])

The variation of this report is significantly related to the value as well as the origin of cultivars, pointing to the fact that there are genetic differentiations in the amino acid composition in the grapevine germoplasm. In order to complete the amino acid profile as a biochemical descriptor, there have been established 3 classes (the class interval = 1.0 mmol) refering to the quantity of amino acid:

Class 3: content $\leq$ 1.00 mmol ; Class 5: 1.01 – 2.00 mmol; Class 7: $\geq$ 2.01 mmol, and 5 classes of the γ ratio (with a class interval of 0.5):

Class 1 :$\leq$ 0,05 ; Class 3 : 0,05 – 1,00 ; Class 5 : 1,01 – 1,50 ;Class 7 : 1,50 – 2,00 ; Class 9 :$\geq$ 2,01

Changes in the amino acid spectrum over the growth and development process have been studied in 1999 by O. Lamikanra and A. K. Kassa on the Noble cultivar of the *Vitis rotundifolia* Michx. (muscadine). Out of the 18 identified amino acids, hystidine was the predominant amino acid, followed by alanine , since the concentration of the predominant amino acids is high at ripening. The glutamine and threonine has suddenly decreased after forming berries, while the arginine and proline concentration has started to increase gradually with ripening. It has been observed that in mature berries, the seeds have the highest amino acid content (50%), followed by the pulp (23%), must (15%) and skin (11%).

Alanine, histidine and arginine have been the main amino acids identified in must and alanine, histidine, arginine, valine, glutamine, asparatic acid, proline, serine and threonine constitute almost 90% of the free amino acids in the pulp.

CONCLUSIONS

1. Researches in this field show a continuous increase in the amino acid content over the ripening process, with a maximum accumulation close to the moment of ripening, due to the stop in the growth of berries and a reduction in protein syntetizing.

2. The total amino acid content and mostly the ratio of predominant amino acids (proline, arginine, glutamine and histidine) differs significantly from one variety of cultivar to another, and even from one year to the next in this species of *Vitis* compared to other species.

3. Observing the spectrum of free amino acids in grapes may be a means to differentiate the grapevine varieties genetically.

4. Is necessary the initiation of research in this area is aiming at verifying and confirming this hypothesis, resulting in the efficient use of the free amino acid profile as a descriptor in identifying the vine genetic material.

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