Aminoacids Profile of cv. Kraljevina (*Vitis vinifera* L.) Clones Candidates

Ivana PUHELEK
Ana Marija JAGATIĆ KORENIKA
Marin MIHALJEVIĆ ŽULJ
Luna MASLOV
Darko PREINER
Ana JEROMEL (✉)

Summary

Different clones from a single grape variety can differ in their productive characteristics and their ability to produce wines with different organoleptic characteristics. The ammonium ions and free amino acids are the main sources of nitrogen for *Saccharomyces cerevisiae* and thus the concentration of nitrogen compounds in the must can significantly affect the wine quality. 'Kraljevina' is well-known Croatian autochthonous variety traditionally grown in Northwest Croatia., year The clonal selection was started in 2003, and in the period 2011-2012 the evaluation of nine clones of 'Kraljevina' grape variety (VV-360,VV-482,VV-486, VV-438, VV-434, VV-479, VV-423, VV-406, VV-483) was conducted. The grapes of the clones tested were harvested on the same day and the obtained juices were filtered and stored for further amino acids analysis. The obtained results pointed to significant diverseness and strong clonal influence on amino acid composition between tested clones. The most abundant amino acid in all clones was arginine ranging from 197.07 to 438.36 mg/L, while the smallest differences were noticed in isoleucine, cysteine, valine and phenylalanine concentrations. The total amino acids concentration ranged from 492.7 mg/L in VV-406 clone to 952.04 mg/L in VV-360 'Kraljevina' clone.

Key words

‚Kraljevina', clonal selection, amino acids, arginine

[1] University of Zagreb, Faculty of Agriculture, Department of Viticulture and Enology, Svetošimunska 25, 10000 Zagreb, Hrvatska
✉ e-mail: amajdak@agr.hr

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Introduction

‚Kraljevina' is an autochthonous grape variety from Prigorje region. Even though it is spread throughout a relatively large area, its name is closely associated with the city Sveti Ivan Zelina and its surrounding area where it is mostly grown. Many quotations confirm that it has been present in the assortment of grape varieties of the viticulture area of the continental Croatia from ancient times and that present the thesis that it is an autochthonous variety (Trummer, 1841; Turković, 1955). ‚Kraljevina' is also known under the name ‚Brina', ‚Imbrina', ‚Kralovina', ‚Dišeća brina', ‚Moravina', ‚Konigtraube' and ‚Portugieser roter'. It is also grown in Slovenia, where it is known as ‚Rdeča Kraljevina', and in Hungary, where it is known as ‚Piros leanyka'.

‚Kraljevina' is only one of authocthonous grapevine varieties of Croatia used in clonal selection for the purpose of the process of revitalisation by means of clonal selection, which has started in 2003. On the territory of Sveti Ivan Zelina, some 100 elite grapevines were propagated into clonal lines, grafted on two virus-free rootstocks and planted on two different locations (Puščak locality and Krčina locality).

The goal of the clonal selection is to extract genotypes with positive characteristics, propagate them and after a long-term evaluation define the clones with better yield, higher grape quality and sugar content, which all leads to better quality of wine as the final product.

Our research was conducted on nine clones of 'Kraljevina' variety that were extracted because of the sufficient number of grapevines that ensured the minimum quantity of grapes required for a proper implementation of the microvinification alcoholic fermentation process. Apart from the basic viticulture characteristics usually used for describing clones (fruitfulness, cluster and berry size, cluster density, etc.), it is very important to pay attention to the quality of grapes, that is, to conduct the appropriate physical and chemical analyses that deal with concrete issues of winegrowers related to the technology and/or quality of their wine. Therefore, in the clonal selection process, it is necessary to examine the variability level of clonal candidates in the synthesis of individual groups of chemical compounds participating in forming sensory characteristics and to define their quality. The chemical compounds include also nitrogen compounds of grape, i.e. must and their relation to alcoholic fermentation and chemical composition of wine. In this sense, the highest attention is paid to the analysis of the amino acid composition. Therefore, the goal of this research was to define the clonal effects on 'Kraljevina' grapevine amino acid profile, and establish to what extent it reflects the genetic differences between individual clonal candidates.

Material and methods

The research was conducted in 2011 and 2012 on the must of candidate clones of ‚Kraljevina' grapevine (*Vitis vinifera* L.) extracted from the grape planted in experimental vineyards located in Sveti Ivan Zelina, more precisely in Gornje Psarjevo, localities Puščak and Krčina, which are typical vineyard locations (Prigorje – Bilogora subregion). The individual vines selected in a positive mass selection within the project "Clonal selection of grapevine cv. Kraljevina" were propagated and planted in both locations. The research was conducted on nine candidate clones of 'Kraljevina' variety (VV-360, VV-438, VV-406, VV-479, VV-486, VV-483, VV-423, VV-434, VV-482) which were selected because of the sufficient number of vines that were able to provide the minimum quantity of grapes required for a proper microvinification alcoholic fermentation process. Each candidate clone was harvested manually after it ripened to full maturity. The grapes were transported in plastic crates to a cellar in Gornje Psarjevo where they were immediately processed. After destemming and crushing of grapes of each candidate clone separately, a 5% sulphurous-acid solution was added to the produced must and the must was cleared by settling for 24 hours, after which time it was poured into 10 L balloon bottles where fermentation was initiated. The must samples were taken for analysis after the must was racked off the lees. Specific amino acids in must were determined by a high performance liquid chromatography (HPLC).

Determination of amino acids in must and wine by HPLC was according to Pripis-Nicolau et al. (2001) with slight modifications. The analyses were performed on an HPLC Agilent 1100 (Agilent Technologies, USA) comprising a binary pump, an autosampler and Agilent 1200 fluorescence detector. The excitations and emission wavelengths were 356 and 445 nm respectively. The column was Lichrospher RP 18 (125 mm × 4 mm × 5 µm). Derivatization of amino acids was made on-line by injection programme of autosampler. The total separation of derivative amino acids lasted 107 min using a flow rate of 0.8 mL/min.

Results and discussion

Besides ammonia, amino acids are the main nitrogen compounds that can be assimilated by yeast (Agenbach, 1977; Jiranek et al., 1995; Kunkee, 1991), and thereby their concentration in must can have a significant effect on wine quality. According to Jiranek et al. (1995), the amino acids that make up the highest percentage of all yeast requirements for assimilable nitrogen include arginine, serine, glutamate, threonine, aspartic acid and lysine. Furthermore, the amino acid profile of *Vitis* varieties has been specified across the entire viticulture and enology world (Huang and Ough, 1991; Kliewer, 1969; 1970; Kluba et al., 1978; Ough and Bell, 1980; Sponholz, 1991), and it has been established that the amino acid composition varies depending on variety and viticulture region. Based on their composition Kliewer (1970) ranked *Vitis vinifere* varieties, and Huang and Ough (1991) established that concentration of specific amino acids varies depending on the year of production. Spayd et al. (1994) pointed out to a significant correlation between the nitrogen fertilization and concentration of specific amino acids.

There are 20 amino acids found in the must and they represent 28 – 39% of the total nitrogen, depending whether the must is made of white or red grape variety (Rapp and Versini, 1991). The concentration of amino acid in the must ranges from 65 to 1130 mg N/1 (Amerine et al., 1980), which primarily depends on variety, region, time of harvest, and nitrogen present in the soil and fertilizer (Bell et al., 1979; Kliewer, 1970). The two most prevalent amino acids available in grape must are proline and arginine and they represent 21 – 55% of total nitrogen. Their concentration is the highest of all amino acids, and they are followed by alanine, asparagine, aspartic acid, glutamate, glutamine, serine, threonine etc. Proline is one of the most abundant

Table 1. Concentration of amino acids (mg/L) in musts of nine candidate clones of 'Kraljevina' grapevine variety, 2011 and 2012 vintage (average value of four samples)

Ak (mg/L)	VV479	VV434	VV423	VV438	VV483	VV482	VV406	VV360	VV486	2011	2012
Glu	20.14^{e}	40.68^{a}	16.68^{f}	24.35^{c}	22.69^{d}	16.84^{f}	17.36^{f}	28.73^{b}	16.12^{f}	21.60^{b}	23.63^{b}
Asp	53.14^{c}	81.96^{b}	38.21^{g}	54.08^{c}	47.30^{d}	45.38^{e}	42.77^{f}	88.03^{a}	38.34^{g}	64.89^{b}	43.93^{b}
Cys	3.42^{c}	5.83^{a}	2.59^{f}	4.16^{b}	3.41^{c}	3.20^{cd}	3.00^{de}	5.62^{a}	2.78^{ef}	6.24^{b}	1.32^{b}
Ser	20.16^{d}	33.18^{b}	18.33^{e}	26.02^{c}	19.99^{d}	25.89^{c}	18.03^{e}	37.63^{a}	17.04^{e}	23.63^{b}	24.42^{b}
His	8.25^{e}	25.56^{a}	8.20^{e}	15.69^{b}	8.66^{de}	11.11^{c}	8.42^{de}	25.34^{a}	8.87^{d}	18.83^{a}	7.85^{b}
Gly	3.95^{f}	8.09^{b}	4.05^{f}	6.14^{d}	5.16^{e}	6.31^{cd}	4.73^{e}	12.60^{a}	6.72^{c}	6.14^{b}	6.69^{b}
Thr	42.75^{g}	73.07^{b}	46.79^{f}	54.34^{d}	51.90^{e}	56.53^{c}	40.85^{g}	83.24^{a}	35.24^{h}	74.00^{a}	33.71^{b}
Arg	231.64^{g}	405.63^{b}	221.32^{h}	277.19^{d}	251.53^{e}	382.11^{c}	197.07^{i}	438.36^{a}	241.98^{f}	414.30^{a}	173.88^{b}
Ala	55.68^{g}	123.72^{a}	72.55^{e}	84.73^{c}	49.65^{g}	78.34^{d}	68.11^{f}	106.83^{b}	72.83^{e}	52.73^{b}	105.59^{a}
Tyr	22.49^{e}	41.34^{a}	23.57^{e}	31.09^{c}	30.17^{c}	30.09^{c}	27.14^{d}	36.01^{b}	25.80^{d}	19.93^{b}	39.56^{a}
Val	3.94^{f}	5.33^{d}	4.55^{e}	5.64^{cd}	7.43^{b}	7.27^{b}	5.52^{d}	8.04^{a}	5.97^{c}	3.87^{b}	8.06^{a}
Met	11.54^{d}	14.70^{b}	11.28^{d}	14.36^{bc}	14.17^{bc}	22.38^{a}	11.63^{d}	21.98^{a}	13.79^{c}	18.02^{a}	12.16^{b}
Phe	2.08^{h}	3.27^{e}	2.93^{f}	4.55^{c}	3.73^{d}	6.74^{a}	3.06^{ef}	5.32^{b}	2.60^{g}	2.62^{b}	5.00^{a}
Ile	3.38^{d}	3.28^{d}	3.24^{d}	3.21^{d}	3.52^{cd}	7.70^{a}	4.55^{b}	7.46^{a}	3.75^{c}	4.93^{a}	3.97^{b}
Leu	11.69^{ef}	12.21^{de}	11.66^{ef}	14.66^{c}	15.10^{c}	21.12^{a}	11.19^{f}	19.41^{b}	12.91^{d}	17.12^{a}	11.76^{b}
Lys	33.80^{b}	48.59^{a}	27.86^{de}	30.00^{c}	29.93^{c}	30.85^{c}	28.89^{d}	27.39^{e}	28.48^{d}	30.01^{b}	33.50^{a}
Σ	528.02	926.50	513.88	650.25	564.41	751.81	492.37	952.04	533.77		

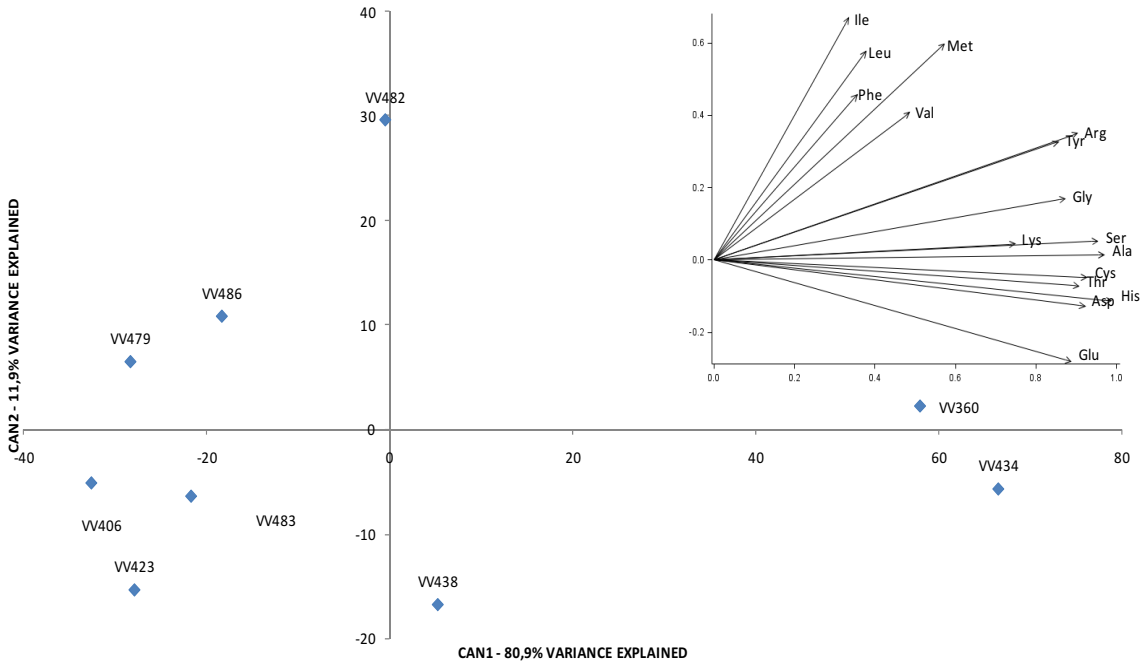

Figure 1.
Results of grouping candidate clones after canonical discriminant analysis in nine must samples for two years of research

amino acids, but it cannot be used by yeasts in typical anaerobic conditions present during alcoholic fermentation. According to some researchers (Agenbach, 1977) a minimum of 140 mg N/L is required so that yeast could fully consume sugar present in must. Research conducted in a synthetic medium has shown the importance of amino acids, especially cysteine, in creation of certain aromatic compounds during vinification process (Pripis-Nicolau et al., 2000).

The composition of amino acids in must is not necessarily identical to the yeast requirements. In such cases, ammonium is primarily incorporated to the molecular structure of insufficient amino acids produced in cells. The use of ammonium salt fertilizers prevents a slowdown or standstill of the fermentation process, and prevents the unpleasant reductive smells.

The chemical analysis of must established significant differences in content of individual amino acids of candidate clones of 'Kraljevina' variety. In both experimental years, arginine was recognized as the most prevalent amino acid with its concentration ranging from 197.07 mg/L in VV-406 clone to 438.36 mg/L in VV-360 clone. The smallest differences in concentration were established in isoleucine, cysteine, valine and phenylalanine. The obtained results are in accordance with the information found in

the relevant literature, which besides proline, specifies arginine as the most abundant amino acid available in must. Therefore, VV-360 clone candidate with its 952.04 mg/L distinguished itself significantly with the highest content of total free amino acids, whereas VV-406 candidate clone with 492.37 mg/L distinguished itself with the lowest content of total free amino acids. Besides arginine, on average, the most abundant amino acids are aspartic acid, alanine, glutamate, lysine, serine, tyrosine and threonine.

In 2011, the majority of amino acids (besides alanine, tyrosine, valine, phenylalanine, and lysine) had a higher average concentration than in 2012. This points to a connection between the concentration of amino acids and agro-technical works in the vineyard, so we can assume that the lack of fertilization, and a lower level of nitrogen matter contained in the soil resulted in a decreased synthesis of amino acids in grapes.

Figure 1 illustrates that VV-360 and VV-434 candidate clones are distinguished from the other candidate clones by their higly positive value of F1 of group centroid that explains 80.9% of the entire variance. These candidate clones stand out with the higher concentrations of glutamate, aspartic acid, histidine, threonine, cysteine, alanine, serine, lysine, glycine, thyrosine, and arganine. The other clones are in the negative section of the coordinate system that infers lower concentration of amino acids.

Conclusion

The obtained results showed that there is a significant difference in amino acid profile between the researched candidate clones and that there is a possibility of using the amino acid profile for the purpose of candidate clone evaluation. Arginine stood out as the most abundant amino acid in both years with concentrations ranging from 197.07 mg/L in VV-406 clone to 438.36 mg/L in VV-360 clone. The least difference was in concentrations of isoleucine, cysteine, valine and phenylalanine. Based on the research results, VV-360 and VV-434 candidate clones were recognized as potentially the most interesting candidate clones, and as the candidate clones that deserve special attention in the further process of clonal selection with the purpose of improving wine quality.

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