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Research on the influence of macroelements on the quality of production in the Victoria variety

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Abstract For the successful marketing of table grapes, berries have to be without imperfections, quality which implies an optimal performance of the vine, the development of bunches and berries but also the preservation of post-harvest quality. Application of mineral nutrients influences the development of the vine, the physiology and berry quality. Despite a large amount of research over the decades, many unresolved issues for ensure optimal quality at harvest and storage of table grapes vines nutrition remain. Fertilization often includes excessive applications of nitrogen (N), potassium (K) and calcium (Ca). For research, an experiment was carried out over four years, on brown-yellow forest soils, using the local variety Victoria training on trellis. As a single product or in combination were applied as fertilizers: nitrogen, potassium and calcium. Depending on the development stage, the dynamics of berry growth also has an impact on the concentration of nutrients in the berries. Berries develop in fast rate, due to cell division and growth which is associated with the rapid decrease in the concentration of nitrogen, potassium and calcium in the berries cells. Due to the higher mobility of potassium (K) and magnesium (Mg) in the plant, the concentration decrease of these two micronutrients, was not as evident compared to other nutrients. The results indicate that the nitrogen (N) in the grapes does not play a key role in the berries sensitivity and damage. Therefore, the late application of nitrogen (N), after the veraison stage, can increase the grapes susceptibility to the diseases attack, especially fungal ones. The potassium (K) content in the berries increased correlated with potassium (K) fertilizer supply. The larger the berry grape is, the larger the skin area and the potassium concentration higher than in the berry flesh.

Key words

grapevine, berries, nitrogen, potassium, calcium

The supply of mineral nutrients affects the growth of plants and their physiology, requiring a balanced intake of mineral nutrients to ensure the performance of the vine and to avoid excessive vigour or mineral deficiencies [3]. Therefore, grapevine nutrition implies mineral composition and an optimal balance between different macronutrients, especially potassium (K) and calcium (Ca) [13]. For grapes, it is generally accepted that some berries qualities, such as the concentration of dry matter, are positively correlated with the berry content in K, while during storage the quality of the fruit is favoured by the low level in nitrogen (N) and calcium (Ca) [25]. In last decades, potassium (K) and calcium (Ca) in soil or foliar application has become common practice for table grapes, even soil is sufficiently supplied. Due to its many functions, many producers consider potassium (K) as a critical nutrient that contributes to colour development and sugar accumulation. Is found in high concentrations in the walls of plant cells, therefore an additional supply of calcium (Ca) is needed to ensure the berries quality after harvest [21, 38]. Nitrogen is one of the most important nutrients in grapevines production and berries quality [2, 11]. For grapevine nutrition deficiency, nitrogen is most commonly applied in vineyards to support optimum growth, but the reaction is different depending on the grape variety [1, 14]. Excessive application of nitrogen fertilizers leads to an increase in the nitrogen in grapes and is also associated with an increased nitrogen content in the vegetative organs and consequently to an excessive vigour [36]. The nitrogen supply has a strong influence on vine size, with impact on the must acidity and increased sensitivity to Botrytis infection [5, 9]. Too much nitrogen lowers the proportion of grape skin and reduces the level of sugar and acidity in the pulp [7, 33]. However, the poor nitrogen supply limits the bunch weight and the titratable acidity, mainly malic acid [8]. Potassium is an essential nutrient for grapevine growing and yield especially when is applied on soil with a high available potassium (K) supply [30, 39]. When there is sufficient potassium supply in soil,

there is no clear response to additional potassium nutrition [15]. During development, grapes are the largest reserve of potassium, especially between veraison and harvesting [20]. The potassium from berries increases during the growing season, reaching the highest concentration at the beginning of the ripening stage [6, 28]. At harvest, grapes contain 66% of the total potassium from the above ground grapevine part [12]. Potassium soil supply can significantly increase the acidity and size of berries, suppress the nitrogen content of the must, and increase the concentration of potassium in the berries skin [26, 35]. Calcium is classified as a secondary nutrient and is also considered the most immobile macronutrient [42]. Although calcium contributes to growth, vines have low requirements for this nutrient [29, 37]. Therefore, calcium deficiencies are rarely found in vineyards [38]. To increase the calcium content in grapes, it is desirable a lower vigour and avoid a dense leaf mass. A favourable effect of pruning in summer was found to reduce the leaf area [31]. The shoots growth is stimulated by fertilization with high doses of nitrogen, and therefore, the calcium stock deviate from the berries to the shoots [16, 42]. During storage, the quality of the fruit is positively influenced by the low level of nitrogen and calcium; therefore nutrition with potassium and calcium has become a common practice in the successful table grape production even there are plenty supplies in the soil [4, 23]. Calcium accumulation in berries occurs mainly in the first stage of development, because after veraison stage starting, calcium accumulates mainly in seeds. Some researchers [19, 41, 43] believe that the decrease of calcium and magnesium concentration in the berries, after veraison stage starting is due to dilution correlated with berry development. Colapietra and Alexander (2006) [10] found in some Italian grape varieties that the foliar application of calcium can promote grape production and sugar accumulation in berries.
Consumers want table grapes with unbroken and uniform berries, a requirement that producers must meet through a management that ensures both high production and quality. Therefore, the research in this paper addresses to the additional fertilization with nitrogen, potassium and calcium in different amounts and ratios, in the Victoria tale grape variety, in order to establish the contribution of these macronutrients to the increase of berries production and quality.

Material and Method

The research was managed over four consecutive growing seasons (2015/16 - 2018/2019) in table grapes Victoria variety (*Vitis vinifera* L.). The plots were planted in 2006. The vine was Geneva double curtain training on 4 wires, with 1.8 m within-row vine space and 3 m inter-row distances, with eight nodes per cane. Analyzes were performed by the Laboratory of Protect Consult SRL.
The experiment was randomized block design. Each experimental plot consisted of five vines in four rows (20 vines). Each treatment was replicated five times. The treatments were combinations of nitrogen (N), potassium (K) and calcium (Ca) different doses applied to the soil, up to 300% of the annual nutritional requirement, while the control treatment represented the standard fertilization applied in commercial production purposes (70 kg N / ha / year, 60 kg K / ha / year, 10 kg Ca / ha / year).
An additional treatment was also included (i.e. additional calcium applied). With the exception of the control plot (nitrogen was applied twice, before lag stage and after harvest, while all amount of potassium was applied after lag stage); the fertilizer was applied manually in six doses throughout the growing season, twice before flowering, three times from lag stage till the veraison and once after harvest. The treatments were applied every year to the same vines.
Berries were sampled several times during each growing seasons, at different days after flowering starting (from veraison to harvest time). From each replication 30 berries were randomly sampled from different bunches. After sampling, berries were weighed and then frozen and stored (in liquid nitrogen at -80°C) for the following analysis (sugar, titratable acidity). Skins were separated from pulp, and separately analysed for main components, including nitrogen, potassium and calcium.
One way ANOVA ($p = 0.05$) was performed for nitrogen, potassium and calcium effects on vines yield and other variables. All statistical analysis of data was processed using GraphPad Prism 7.04.

Results and Discussions

Successful table grapes growing suppose good quality bunches and favourable storage conditions after harvest. It is generally accepted that some variables of grape quality, such as sugar concentration, are positively correlated with the potassium level from berries [18, 27]. For fresh consumption, table grapes are harvested at lower sugar concentrations than wine grapes. Therefore, the study focused on the accumulation of nutrients until early maturity, which is about 120 days of growth.
High nitrogen supply contribute to vigorous vines and shading canopy, but in the same time increase the berry size which finally reduce the grape skin to pulp ratio [16, 32]. The nitrogen concentration from berry pulp, which represents 60 - 90% of the total nitrogen from grape berries decreases rapidly, beginning from the lag stage to the veraison stage, and after that the decrease is slowed down. The concentration of nitrogen from the berries skin follows a similar trend. The total accumulation of nitrogen in berries was fast in the previous stage before veraison, activity correlated with cell division and growth, when nitrogen is necessary for chlorophyll, nucleic acids and proteins according to

Löhnertz et al. (2000) [28]. After veraison, the accumulation of nitrogen slows down which probably explains the increase in the concentration of nitrogen in the berry skins from the samples collected in the 2018/19 growing season. During 2015/2016, 2016/17 and 2017/2018 growing seasons was recorded a significantly higher increase in berry weight compared to the control, as response to the nitrogen supply.

However, for five samples taken in all four seasons, berry from plots fertilized with nitrogen or combination between nitrogen and calcium, showed the highest weight (Table 1). The results are different from those reported by Ruiz et al. (2004)[40] which indicated a strong correlation between the accumulation of potassium and the berries weight in wine grape varieties.

Table 1

Influence of nitrogen, potassium and calcium fertilization on berry weight (g) in Victoria variety

Plot / treatment	Berry weight (g)						
	2015/2016	**2016/2017**	**2017/2018**		**2018/2019**		
	81 days*	73 days*	68 days*	78 days *	67 days*	74 days*	88 days*
Control	7.72ab	6.68b	9.22	8.78ab	7.24ab	7.73ab	7.37
Ca (Bunch)	7.33b	6.73b	9.18	8.86ab	7.27ab	7.49b	7.90
N	8.38a	7.43a	9.22	9.87a	7.80ab	8.46ab	7.94
K	7.67ab	6.82ab	9.25	8.49b	7.68ab	8.03ab	8.15
Ca	7.32b	7.04ab	9.36	8.81ab	6.96b	8.02ab	7.74
KCa	7.83ab	6.37b	9.55	8.42b	7.63ab	8.15ab	8.56
NCa	8.26a	6.56b	9.13	8.55b	8.04a	8.30ab	8.52
LSD ($p \leq 0.05$)	**0.79**	**0.67**	**NS**	**1.16**	**0.79**	**0.82**	**NS**

* Days from the beginning of flowering; ab - the difference between the averages in the same column

Some experts [2, 14, 17, 35] believe that soil composition has an influence on the effect of nitrogen fertilization on vines, on the quality of grapes and berries, respectively. Bell and Robson (1999) [1] stated that nitrogen fertilization on sandy soils and low organic matter favoured the yield increase. El-Razek et al. (2011) [22] found that single berries of Crimson Seedless variety were bigger (weight and size) by increasing nitrogen nutrition, while the berry shape was not affected. In similar research, Habran et al. (2016) [24] found in Pinot Noir variety that nitrogen supply increased amino acids from skin and berry pulp.

The effect of fertilization variants on the berries sugar level in the Victoria variety was variable during all four growing seasons. However, in both plots, - the control and fertilized with potassium – there is a trend to have the highest sugar content in the berries (Table 2). The small positive effect of potassium fertilization on berry sugar content is in line with the results of Conradie (1992) [14] which found that fertilization with potassium up to 90 kg / ha slightly increased the sugar content compared to the control.

Table 2

Influence of nitrogen, potassium and calcium fertilization on berry sugars content (^{o}Brix) (Victoria variety)

Plot / treatment	Sugars (^{o}Brix)					
	2015/2016	**2016/2017**	**2017/2018**		**2018/2019**	
	81 days*	74 days*	68 days*	77 days*	74 days*	87 days*
Control	19.21	15.11a	14.02ab	16.11a	18.52a	19.61
Ca (Bunch)	17.62	14.21ab	14.21ab	15.60ab	17.04ab	18.43
N	17.89	14.23ab	13.51b	15.42ab	16.12ab	18.14
K	19.11	15.11a	14.49a	15.59ab	16.21ab	19.32
Ca	18.21	14.89a	14.12ab	15.33ab	17.13ab	19.44
KCa	16.92	15.12a	14.03ab	15.51ab	16.12b	19.26
NCa	17.72	13.41b	13.21b	14.42b	17.14ab	18.61
LSD ($p \leq 0.05$)	**NS**	**1.09**	**0.78**	**1.1**	**1.4**	**NS**

* Days from the beginning of flowering

Even potassium is one of the macronutrients required by grapevine, only few studies mentioned an increase of bunches or /berries weight. According to Brunetto et al. (2012) [6], the potassium effect is stimulated when is combined with nitrogen. There is a strong relationship between the potassium level and the accumulation of sugar and dry matter in berries. El-Razek et al. (2011) [22] reported significant increase of sugars/acid ratio after increasing amount of potassium fertilization, in Crimson seedless variety. On contrary, nitrogen fertilization did not have a well-defined effect on berries sugar content in none of the four growing seasons.

During the 2017/2018 and 2018/2019 growing seasons, additional fertilization largely influenced the sugar content in berries, in a rate of about 70%. In all four growing seasons, additional fertilization, either in soil or foliar fertilization, led to the accumulation of a different amount of sugar in the grapes and due to the climatic conditions of each growing season, given that the amount of fertilizer used was the same every year.
The titratable acidity decreased from sample to sample (Table 3). The variant in which potassium fertilization was applied showed the highest titratable acidity (TA) content for the first and second sampling, but nevertheless was not significantly higher than in the control variant.
By applying nitrogen or calcium fertilization there was no constant effect on the titratable acidity. Increased acidity may be the consequence of a denser canopy due to nitrogen fertilization, which leads to a higher shading of bunches, compared to unfertilized vines. Therefore, excessive vigour reduces the optimal conditions for photosynthesis, which induce titratable acidity decreasing.

Table 3

Influence of nitrogen, potassium and calcium fertilization on titratable acidity (g /l), in the Victoria variety

Plot / treatment	Titratable acidity (g/L)				
	2015/2016	**2017/2018**		**2018/2019**	
	81 days*	**68 days***	**79 days ***	**74 days***	**88 days***
Control	2.98ab	3.51	3.04a	3.57ab	2.97abc
Ca (Bunch)	2.88ab	3.49	2.92ab	3.35abc	2.88bc
N	2.72b	3.76	2.68ab	3.33abc	2.77cd
K	3.27a	3.64	2.66ab	3.82a	3.08ab
Ca	2.75ab	3.46	3.02ab	3.12bc	2.63d
KCa	2.97ab	3.70	3.04ab	3.05c	3.15a
NCa	-	3.58	2.58c	-	-
LSD ($p \leq 0.05$)	**0.54**	**NS**	**0.35**	**0.47**	**0.23**

Climate had a major influence on vines growth and development during 2017/2018 and 2018/2019 growing seasons. In the first year in which the titratable acidity was measured, the additional fertilization had an influence of almost 70% on the acidity of Victoria grapes before the harvest stage, while during last growing season (2018/2019), the additional fertilization had minor influence (13%) on berries acidity. According to Omar (2000) [34] research results, high potassium fertilization of Thompson seedless grape reduced titratable acidity.
Skin and pulp berries samples, from each growing season, were analysed separately for macronutrients composition.

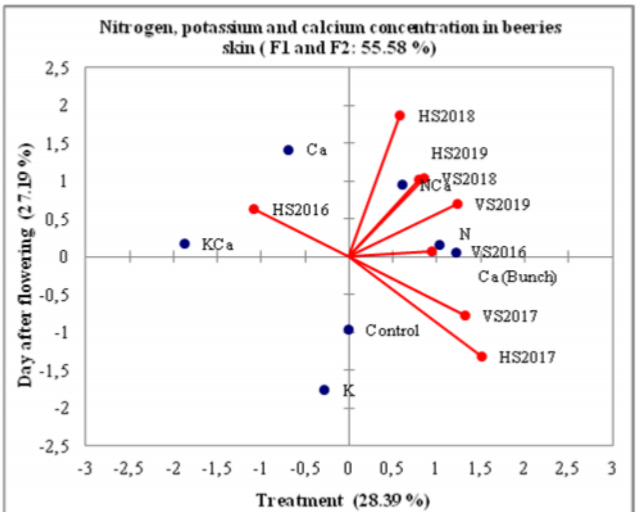

Figure 1

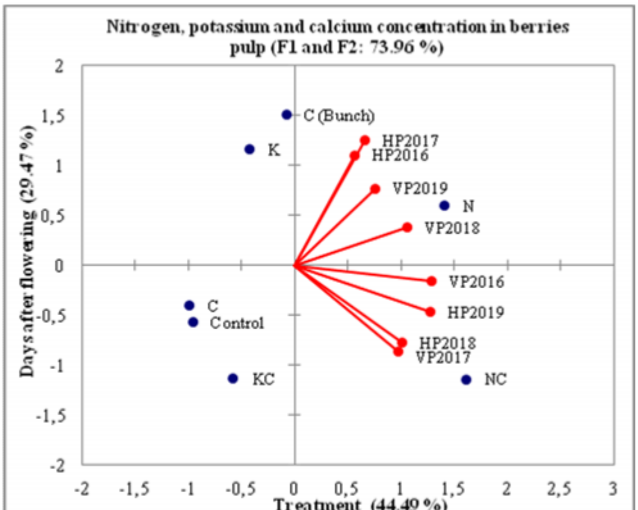

Figure 2

In Principal Component Analysis (PCA) biplots are presented the concentration of nitrogen, potassium and calcium application in berries skin (figure 1) and pulp (Figure 2). PCA analysed variance within groups trial data and between groups. Variables include fertilizers and different intervals of days after flowering.

Abbreviations: VS=Veraison skin (sample were collected during veraison stage); HS = harvest skin (sample during first harvest); VP = veraison pulp (sample were collected during veraison stage) and HP = harvest pulp (sample during first harvest).

Conclusions

Depending on the development stage, the dynamics of berries growth had an impact on the concentration of nutrients. Grape berry growth in a fast rate, due to cell division and growth, is associated with the rapid decrease of nitrogen, potassium and calcium concentration in berries. The nutrients concentration is expected to have high impact on berries quality due to the differences that exist between the growing seasons, which are influenced by climate variability, which lead to a variation in berries growth and size, as well as to the accumulation of sugar. Larger berries after nitrogen application are attributed to early vegetative growth. Both the application of potassium and the combination of potassium and calcium had a slightly positive effect on the sugar content in berries, in the Victoria table grape variety.

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