Bulgarian Journal of Agricultural Science, 23 (No 3) 2017, 389–395
Agricultural Academy

PHENOLIC COMPOSITION OF VRANEC GRAPEVINE CULTIVAR (*VITIS VINIFERA* L.) GRAFTED ON DIFFERENT ROOTSTOCK

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Abstract

Nedelkovski, D., J. Cvetković, K. Beleski and H. Poposka, 2017. Phenolic composition of Vranec grapevine cultivar (*Vitis vinifera* L.) grafted on different rootstock. *Bulg. J. Agric. Sci.*, 23 (3): 389–395

The objective of this work comprises the content of phenolic compounds in grape and wine produced by Vranec grapevine cultivar grafted on four different rootstocks (Teleki, Fercal, Rupestris Du Lot and Chasselas 41B). Total phenols, total flavan-3-ols, total anthocyanins and total flavonoids in the grape berries, separately in seeds, pulp and skin were determined. Three months later, the same parameters were analyzed in the wine obtained by the investigated grape variety. The field investigations showed that Teleki and Fercal were more vigorous rootstocks than Rupestris Du Lot and Chasselas 41B, but there was not significant deference in the phenological stages between the rootstocks. The grape clusters of Vranec grafted on Fercal and Teleki were larger than Rupestris Du Lot and Chasselas 41B. The results obtained from the laboratory analysis showed higher level of total phenols in the berries of Vranec grafted on Teleki rootstock, higher level of total anthocyanins in the berries of Vranec grafted on Chasselas 41B rootstock, higher level of total flavonoids in the berries of Vranec grafted on Rupestris Du Lot rootstock and the higher level of total flavan-3-ols in the berries of Vranec grafted on Fercal rootstock. Statistical analyses were performed using SPSS software 14.0. The differences were evaluated by ANOVA analysis through General Linear Model (GLM) procedure. After GLM analyses post hoc comparison of means were calculated by LSD (Least Significant Difference). Results were expressed at the $P = 0.05$ level of significance.

Key words: Vranec grapevine; total phenols; Chasselas 41B; Teleki rootstock

Introduction

Grapes *(Vitis vinfera* L.) are considered the world's most prevalent fruit crop. Their large amounts of phenolic compounds have made them the focus of extensive studies (Broussaud et al., 1999; Caillet et al., 2006; Bozan et al., 2008). In grape berries, the phenolic compounds reside mainly in the skins and seeds (Rodriguez et al., 2006; Poudel et al., 2008). They undergo partial extraction during the winemaking process. Phenolic compounds play an important role in the quality of grapes and wines. They can be divided into two groups: non-flavonoid (hydroxybenzoic and hydroxycinnamic acids and stilbenes) and flavonoid compounds such as anthocyanins, flavan-3-ols and flavonols (Rodriguez et al., 2006). Anthocyanins are a family of phenolics that are directly responsible for color in grapes and young wines. They may react with flavanols to produce more stable pigments, either directly or by means of different aldehydes (e.g. acetaldehyde, propionaldehyde) (Pisarra et al., 2003). Flavan-3-ols (monomeric catechins and proanthocyanidins) are another large family of phenolic compounds that are mainly responsible for the astringency, bitterness and structure of wines. Finally, phenolics, particularly certain phenolic acids, participate in the phenomenon of co-pigmentation. The last group of flavonoids is flavonols quercetin, myricetin, kaempferol, isorhamnetin and their glycosides, which are potent antioxidants. Phenolic compounds in grapes and wine have attracted much

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interest due to their antioxidant properties (Kanneret al., 1994; Llobera and Canellas, 2007) and their potentially beneficial effects on human health (Teissedre et al., 1996; Vitseva et al., 2005).

Vranec is a dark-skinned variety of grape. In the Republic of Macedonia it is dominant grape variety among the red varieties. This variety is widely spread in the other Western Balkan countries (Montenegro, Bosnia and Herzegovina, Croatia and Serbia) and is used primarily to produce excellent red wines, which enjoy great popularity on the marketplace. Winemaking in Macedonia is traditionally based on local varieties such as Vranac due to its lower heterogeneity and improved skin coloration. Vranec grapes are considered to have a strong polyphenol (Ivanova et al., 2011), as well as a high colour potential (Avramov, 1991).

The objective of this study is to examine the influence of the rootstock on the quality of the Vranec grape berries regarding the total phenols, total flavan-3-ols, total anthocyanins and total flavonoids, as well as their impact on the phenological characteristics on the grape vine. In order to improve the quality of the grape, but also to achieve better resistance to some pests, nematodes and disease, vine grapes were crafted on four different rootstocks: Teleki, Fercal, Rupestris Du Lot and Chasselas 41B. The results obtained, regarding the quality of the grape, are presented in this study. The grapes harvested from different rootstocks were used for wine production in the experimental laboratory of the Institute of Agriculture.

Materials and Methods

Field investigations

The field investigations were performed in the experimental vineyard of the Institute of Agriculture, Skopje. Vranec grape vine variety was grafted on four different rootstocks (Teleki, Fercal, Rupestris Du Lot and Chasselas 41B). The pruning system was double guyot, two canes with eight buds and two spurs with two buds, which is 20 buds per grape vine in total. During the investigation, standard agricultural procedures were applied.

Yield and mechanical composition of the grapes

The grape was harvested at the end of September (late harvest) which is the main reason for the relatively high amount of sugar (approximately 24-26^{o} Brix). The yield and the mechanical analysis of the grape clusters and the grape berries were performed in the experimental laboratory in the Institute of Agriculture, using standard methods (Avramov, 1991).

Vinification

Processing of grape was performed in the microvinification cellar, according to the standard procedure for making red wines. The grapes were harvested manually. For the vinification an average grape sample of each yield was used. In order to prevent oxidation and for microbiological protection, potassium metabisulfite was added (10 g per 100 kg of grapes). All enzymes, wine yeasts and yeast nutrients were obtained from Lallemand, Australia. Lallzyme EX V (1 g per 100 kg of grapes) for maceration was added during red wine vinification. Yeast Lalvin D254 was used for each variety. Yeast nutrient, Fermaid E (25 g per hL) was added during fermentation and Go-ferm protect (30 g per hL) was used for yeast preparation.

Spectrophotometric analyses of grape berries and wine

Extraction procedure

A selective extraction of polyphenols from the skins and seeds of grape berries that simulates the maceration process of red wines was used (Llobera and Canellas, 2007). Skins and seeds of 100 g randomly sampled grape berries were manually separated and separately extracted in a solution consisting of methanol:water:HCl (20:79.9:0.1). Extracts were poured into dark glass bottles, flushed with nitrogen and stored at 4°C until required for spectrophotometric analyses.

Spectrophotometric determinations were carried out on the extracts according to the methods of Di Stefano and Cravero, 1991. Spectrophotometric analyses of wine were performed by direct measurements or using appropriate dilution of wine in distilled water when necessary.

- ***Total phenols*** content were assessed by the reduction of Folin-Ciocalteu reagent. The results obtained are expressed as mg/L gallic acid equivalent (GAE/L).
- ***Total flavonoids*** were measured using a colorimetric assay developed by Dewanto et al. [14]. An aliquot of diluted sample or standard solution of (+)-catechin was added to *75* μL of $NaNO_2$ solution (7%) and mixed for 6 mm, before adding 0.15 mL of $AlCl_3$ (10%). After 5 min, 0.5 mL of 1 M NaOH solution was added. The final volume was adjusted to 2.5 mL and mixed thoroughly, and the absorbance of the mixture was determined at 510 nm. Total flavonoids were expressed as mg (+)-catechin equivalent (CE) per g fresh weight basis (mg CE/g), through the calibration curve of (+)-catechin.
- ***Extractable anthocyanins*** were quantified on the basis of maximum absorbance in the visible light interval of 536 to 540 nm and calculated as malvidin (mg/kg of grape). Similar procedure was applied for determination of total

anthocyanins in wine. Wine dilution (1:100) was added in 10 ml flask and made up to volume with a solution of ethanol:water:hydrochloric acid (70:30:1). The absorbance measured at 536 to 540 nm against ethanol chloride as blank was used for calculation the concentration of total anthocyanins (TA) in wines using the equation:

$$TA_{540nm}(mg/L) = A_{540nm}16.7d,$$

where $\mathbf{A_{540nm}}$ is the absorbance at 540 nm and **d** is the dilution factor.

● ***Total flavan-3-ols*** were determined using DMCA *(p*-dimethylaminocinnamaldehyde) method. An aliquot of 0.1 mL of wine was purred in a 10 mL flask, than few drops of glycerol and 5 mL DMCA solution were added and made up to volume with methanol. The absorbance at 640 nm was measured against methanol as blank.

Statistical analysis

Statistical analyses were performed using SPSS 14.0 (SPSS Inc., 2005). The differences were evaluated by ANOVA analysis through General Linear Model procedure. After GLM analyses post hoc comparison of means ware calculated by LSD. Results were expressed at the P = .05 level of significance.

Results and Discussion

Yield and physicochemical composition of the grapes

The comparisons of different grafting combinations for the variety Vranec are presented in Table 1. The highest yield was obtained with rootstock Teleki (2990 ± 402.8 g/vine) which is significantly different from other combinations. This combination produced more than 700 g/vine higher yield compared with the combination Vranec/Rupestris Du Lot. The influence of rootstocks on the yield has been reported by many studies (Kocsiset al., 2012; Pulkoet al., 2012; Butkhupl et al., 2010; Toit and Visagie, 2012).

In agreement with the highest yield, Vranec/Teleki combination showed highest cluster weight (295 ± 79) but lowest weigh of 100 berries (165 ± 4.6), due to the smaller berries. Another positive characteristic for this combination is the lowest number of seeds in 100 berries (196 ± 8.3) compared with the other investigated combinations (which ranged from 200 to 240). The results concerning the grape mechanical composition also showed that Rupestris Du Lot rootstock, compared with other rootstocks, resulted in a lower average weight of the cluster. On the other side, the weight of 100 berries on Rupestris Du Lot rootstock was 10.2-21.8% higher in comparison to rootstocks Fercal, Chasselas 41B and Teleki.

In general, the yield and the physicochemical composition of the grapes demonstrate the compatibility between the rootstocks and the soil. Different rootstock have affinity to different soil type. Some authors (Bozinovic, 1996; Roichev, 2012) suggest that rootstock Chasselas 41 B in general has good affinity to all soil types that are not badly drained wet soils, such as clay cold soils. Chasselas 41B is known as rootstock with good resistance to carbonates. Fercal is characterized with very good developed deep root system, which is suitable for micro regions poor with rainfalls. This rootstock has very high tolerance to carbonates. Rupestris Du Lot is rootstock for deep water releasable soils (sandy) in which the roots are developed very deep. This rootstock is not so resistant to carbonates. Teleki rootstock is suitable for moderately wet, drained carbonate soils.

The experimental vineyard with the investigated variants was established on clay soil. This type of soil is described as soil with high level of carbonates and ability to hold the water (Filipovski, 1993). According to some authors (Whiting,

Table 1
Yield and physico-chemical grape composition (± SD) of Vranec variety from different rootstocks (n = 5)

Parameter	Rootstocks			
	Teleki	Fercal	Rupestris Du Lot	Chasselas 41B
Yield (g/vine)	2990 ± 403[a]	2770 ± 293[b]	2255 ± 331[d]	2600 ± 346[d]
Cluster weigh (g)	295 ± 79[a]	246 ± 68[b]	211 ± 32[b]	235 ± 29[b]
Grape stem weight (g)	8.0 ± 0.2[b]	7.5 ± 0.2[c]	8.5 ± 0.3[a]	8.0 ± 0.2[b]
Weight of 100 berries (g)	165 ± 4.6[c]	190 ± 2.8[b]	212±5.3[a]	189 ± 3.1[b]
Seed number/100 berries (g)	196 ± 8.3[c]	240 ± 6.8[a]	230 ± 5.5[b]	200 ± 12[c]
Seed weight/100 berries (g)	3.0 ± 0.1[c]	3.1 ± 0.1[b]	3.1 ± 0.1[b]	3.2 ± 0.2[a]
pH	3.52 ± 0.09	3.47 ± 0.07	3.48 ± 0.07	3.25 ± 0.05
Titratable acids (g/L tartaric acid)	5.5 ± 0.2	6 ± 0.3	5.5 ± 0.2	6.3 ± 0.4
Total soluble solids (°Brix)	26.0 ± 0.5	26.5 ± 0.6	26.0 ± 0.6	24.5 ± 0.4

Data followed by different letters within each column differ significantly at P = 0.05

2003, Clarke, 2004) vines grafted on Rupestris Du Lot compered to Teleki have lower yield and smaller berries. This is in agreement with our results.

Phenolic composition of grape berries

Phenolic composition of Vranec grape berries grafted on different rootstocks, at the time of harvest, is shown in Table 2.

The mean content of ***total extractable phenols*** in the skin of the grape berries was the highest in Vranec/Rupetris Du Lot combination (19.3 mg/g), which is significantly higher, compared to Vranec/Fercal combination (16.0 mg/g). The statistical analysis of total phenols for the grape berry pulp showed statistical differences between Shasla 41B, Teleki 5C and Fercal. The combination Vranec/Teleki showed the lowest amount of total phenols (0.89 mg/g), compared to other combinations. The seed analysis for total phenols showed similar amounts of these compounds at Vranec/Fercal and Vranec/Fercal combinations, but significantly different amount at Vranec/Rupetris Du Lot combination, which is lowest (73.3 mg/g). This combination has highest amount of total phenols in skin, which indicates that phenol distribution is slightly different than in the other combinations of Vranec/Rootstocks.

Total anthocyanins were detected in the skin and the pulp of the berries. As expected and mentioned by many authors (Ivanova et al., 2011; Butkhupl et al., 2010) seed does not contain anthocyanins and the pulp is poor with these compounds. The amount of anthocyanins showed clear significant differences between the combinations ($P = 0.05$). As presented in Table 2, highest concentration was observed in berry skin of Vranec/Teleki combination, which is 65% higher than those found in Vranec/Chasselas 41B combination. Statistical differences were not found only between the Vranec/Fercal and Vranec/Rupetris Du Lot. The content of total anthocyanins in the grape berry pulp ranged between 0.50 and 0.61 mg/g. Significant statistical difference between the examined pulp samples was found between the rootstocks Fercal and Teleki and also between Teleki and Rupestris Du Lot.

The data for the ***total flavonoid*** contents showed that these compounds are mainly present in the seeds and less in the skin of the grape. The results presented in Table 2 show that seeds from Vranec/Fercal combination contain statistically proven highest amount of total flavonoids (44.29 mg/g) compared to all other combinations.

In their richness with phenolic components, red grape skins and seeds contain some secondary compounds that are important for their antioxidant activity, such as ***flavan-3-ols.*** Considering the berry skin, the results presented in Table 2 shows that there is significantly lowest amount in the combination Vranec/Fercal compared to other combinations. All other combinations showed similar concentration of flavan-3-ols. Yet, the seeds contain pretty high amount of these compounds. The analyzed seed samples showed statistically proven difference between all the rootstocks, except between Rupestris Du Lot and Teleki. These findings are consistent with previous reports relating to grape varieties grown in the region and around the world (Negro et al., 2003; Ivanova et al., 2010).

Table 2
Phenolic composition of Vranec grape berries on different rootstocks

	Total phenols, mg/g GAE	Total anthocyanins, mg/g	Total flavan-3-ols, mg/g	Total flavonoids, mg/g
Teleki				
Skin	18.7^{b}	16.1^{a}	1.04^{a}	3.90^{b}
Pulp	0.89^{c}	0.43^{b}	traces	traces
Seed	80.3^{b}	NA	19.6^{c}	40.1^{ab}
Fercal				
Skin	16.0^{c}	13.1^{b}	0.77^{b}	3.57^{b}
Pulp	1.15^{b}	0.61^{a}	traces	traces
Seed	82.3^{a}	NA	20.9^{b}	44.3^{a}
Rupetris Du Lot				
Skin	19.3^{a}	13.6^{b}	1.06^{a}	3.95^{b}
Pulp	1.73^{a}	0.58^{a}	traces	0.12
Seed	73.3^{c}	NA	19.9^{c}	36.7^{b}
Chasselas 41B				
Skin	19.0^{a}	10.4^{c}	1.09^{a}	$4{,}62^{a}$
Pulp	1.72^{a}	0.50^{ab}	traces	traces
Seed	82.7^{a}	NA	24.5^{a}	38.9^{ab}

Data followed by different letters within each column differ significantly at $P = 0.05$

Wine phenolic composition

The levels of the different classes of phenolic compounds in wine are subject to a large variability. The phenolic composition depends on grape variety, but also on additional factors, such as climate and even on vine location in the same vineyard, enological practices, the storage conditions, etc (Bubola et al., 2011). Grape seeds and skin are the main source of phenolic compounds which determines wine color and structural properties.

The process of vinification enables rapid, partial extraction of grape phenolics into dilute alcohol solution, but low pH and the presence of yeast-derived metabolites also initiates their conversion into derivatives, formation of noncovalent associations and oxidative degradation (Cheynier et al., 2006).

Influence of rootstocks on phenolic compounds in wine

The results from the analysis of the phenolic composition of the three-month old wines produced by grape harvested on different rootstocks are presented in Table 3. It is obvious that rootstocks significantly influence the concentration of several phenolic compounds in finished young wines.

As presented in Table 3, highest gallic acid was recorded on Vranec/Fercal combination (3744 mg/L GAE) and lowest in Vranec/Chasselas 41B (2483 mg/L GAE). Though presence of total phenolic compounds in grapes grafted on Fercal rootstock was not the highest, the best extraction was obtained after fermentation, compared to other rootstocks. The phenolic profile in the wine largely depends on the total phenols present in fresh grapes, such as the extraction parameters, wine making technologies and other chemical reactions taking place during fermentation process (García-Falcón et al., 2007; Fang et al., 2008). It is worth to be mentioned that all investigated wine samples were analyzed three months after vinification (yang wines), which justify the high amount of phenolic compounds, compared to the published results for other world red wines (Broussaud et al., 1999; Rodriguez et al., 2006; Bozan et al., 2008; Poudel et al., 2008).

Similar behavior was observed with the amount of total anthocyanins. Vranec/Fercal combination contains significantly higher amount of these compounds (483 mg/L) compared to Vranec/Rupetris Du Lot and Vranec/Chasselas 41B combination (355 mg/L and 314 mg/L, respectively), but similar with Vranec/Teleki.

Flavonols constitute a small portion of the phenolic compounds in wine. The overall quantity is influenced by factors such as the variety, maceration process and climatic conditions (Sipiora and Gutiérrez Grande, 1998). Despite their low concentration, they are important since they may participate in the co-pigmentation phenomenon with anthocyanins, changing wine color and stabilizing pigments (Jogaiah et al., 2015). Significantly highest amount of total flavonoids was detected at Vranec/Fercal wine (1751.62 mg/L) compared to all other combinations. Flavan-3-ols are tannins which are mostly located in grape skins and seeds. Extraction of tannins from seed depends on type of yeast culture used, fermentation temperature, alcohol content etc. Usually, flavan-3-ols which are located in seeds are protected with a lipidic layer. This layer can be disrupted when concentration of alcohol increases, allowing their extraction from seeds (Ivanova et al., 2009). Concerning flavan-3-ols, only (+)-catechin was quantified in the investigated wines. Compared to other worldwide wine analysis (Cheynier et al., 2006; García-Falcón et al., 2007), its content was relatively high and different among varieties, and ranged from 305 to 501 mg/L. Previous investigations (Ivanova et al., 2010; Ivanova et al., 2011), confirmed the high influence of the yeast on catechin content probably because of a lower adsorption of catechin on the yeast cell walls at some yeasts.

Conclusions

From the present study, it can be concluded that the grapes harvested on different rootstocks showed significant difference between their ability to perform best results regarding yield, physicochemical and phenolic grape composition, as well as phenolic composition of wine.

As presented in this study, best yield and physico-chemical properties of grapes were found at Vranec/Teleki combination. However, concerning the phenolic composition of grape berries, different trend was observed. Highest amount

Table 3
Influence of rootstocks on phenolic compounds of Vranec wines

Rootstock	Total phenols, mg/L GAE	Total anthocyanins, mg/L	Total flavan-3-ols, mg/L	Total flavonoids, mg/L
Teleki	3233^{b}	468^{a}	451^{b}	1520^{b}
Fercal	3744^{a}	483^{a}	501^{a}	1751^{a}
Rupetris Du Lot	2753^{c}	355^{b}	311^{c}	1089^{c}
Chasselas 41B	2483^{d}	314^{b}	305^{d}	1113^{c}

Data followed by different letters within each column differ significantly at P = 0.05

of total phenols was found in the seeds at Vranec/Fercal and Vranec/Chasselas 41B combinations. Furthermore, wines prepared from grapes harvested on Vranec/Fercal rootstocks also showed higher total phenolic content than on other rootstocks, but wines prepared from grapes harvested on Vranec/Chasselas 41B rootstocks, unexpectedly, showed lowest total phenolic content. Obviously, better extractability was achieved in the former case.

Highest amount of total anthocyanins were found in the skin from berries harvested from Vranec/Teleki vines, as well as in the wine produced from this grape. The extractability of anthocyanins is very important characteristic, though it is well known that the anthocyanins content of wine can be affected by the yeast during fermentation. Since the stability of the wine color largely depend on the anthocyanins amount, the yeast choice is very important. However, the process of fermentation which involves phenolic extraction, conversion and potentially significant losses of extracted phenolics is a complex process, with anthocyanin extraction limited by unknown factors. As a result, a strong correlation between grape and wine phenolic composition was not observed. Therefore, no conclusion can be drawn regarding the grape polyphenol potential of the investigated rootstocks. For certain results and conclusion, a multiyear study will be carried out in the future to evaluate the polyphenol potential of grapes from different rootstocks and vineyard locations.

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Received March, 17, 2017; accepted for printing May, 2, 2017