Scientia Horticulturae 237 (2018) 74–80

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Impact of clonal selection on Cabernet Franc Grape and wine elemental profiles

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ARTICLE INFO

Keywords:
Cabernet Franc
Clonal selection
Grape
Wine
Soil
Elemental profile
ICP-OES

ABSTRACT

Three Cabernet Franc (CF) clones (Nos. 02, 010 and 012; not yet commercial) obtained in the last phase of clonal selection were examined within this study. Indeed, the content of 27 chemical elements in the vineyard soil along with CF grape and wine samples were determined by inductively coupled plasma - optical emission spectroscopy. The relative dependence of the selected elements in all samples were estimated using correlation matrices. While elemental profiles of both CF grape and wine samples were highly variable among the analysed clones, bioaccumulation factors (grape/soil) of Mg, Na and Sr were found to be specific for each single clone. Applying principal component analysis, the grape clones were differentiated among each other according to the content of 10 elements (Al, B, Ca, Cu, Fe, K, Mg, Mn, Na and Sr). Finally, hierarchical cluster analysis of CF grape and wine samples pointed out the similarity of the clones Nos. 010 and 012, since they were grouped within the same subcluster.

1. Introduction

Grapevine (*Vitis vinifera* L.) is widely grown plant around the world. Nowdays it globally represents the most important fresh fruit crop (Đorđević et al. 2018; Marsal et al. 2017; Vujovic et al., 2016; Vujović et al. 2017). Apart from economy, health promoting effects significantly contribute to its importance (Jackson 2008). In Serbia, both viticulture and enology do have a long tradition that dates back to the Roman times. Undoubtedly, Cabernet Franc (CF) belongs to the most significant red grape varieties within the country (Pantelić et al. 2016). Though originated from France, this variety has been successfully introduced in a number of regions with different climate (Cindric et al. 1996). It is involved in the parentage of prominent varieties such as Cabernet Sauvignon and Carménère (Pantelić et al. 2016). CF clonal selection is a very important tool for obtaining the clones to be used for the production of high quality wines (Cindric et al. 1996). Initially, the major aim of the clonal selection was to create a virus free population from healthy mother plants (Lacombe et al. 2004). Later on more complex selection criteria have been applied (van Leeuwen et al. 2013). For a variety with great genetic diversity, its clonal selection represents a major issue affecting the wine quality (Zamuz et al. 2007). On the other hand, nutritional values and organoleptic characteristics of both grapes and wines partially depend on their elemental contents (Geana et al. 2014).

The elemental content of grapes and wines is influenced both by various natural (vineyard soil and climate) and artificial (environmental pollution and vinification) sources (Almeida & Vasconcelos 2003; Castiñeira et al. 2004; Geana et al. 2014; Cozzolino 2015; Đurđić et al. 2017). Potassium, calcium, iron and copper may produce precipitates that affect organoleptic properties of the wine, particularly its visual characteristics. Additionally, this content may be of help in its distinguishing and typifying followed by geographical discrimination, contributing in such a way to the avoidance of fraud (Frías et al., 2001; Thiel et al. 2004; Bertoldi et al. 2011). Finally, such kind of study is useful for checking out the wine quality (Pohl 2007; Grindlay et al., 2008; Đurđić et al. 2017).

Our previous work on the subject of this study – CF grape and wine clones – has revealed significant differences in chemical composition and anti-DPPH radical activity among the grape samples (Pantelić et al. 2016; Pejin et al. 2016; Popovic-Djordjevic et al. 2017). Actually, the

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https://doi.org/10.1016/j.scienta.2018.03.043
Received 3 February 2018; Received in revised form 17 March 2018; Accepted 20 March 2018

grape of CF clone No. 010 possessed an icreased content of polyphenolics than other two CF clones. In addition to this, the wine of the same CF clone was found to be distinguished in a variety of parameters, compared both to the mother clone wine and two remaining clones, Nos. 02 and 012. Indeed, the wine of the clone No. 010 contained the highest concentration of aldehydes, esters, anthocyanins, polyphenolics and resveratrol. Consequently, the same wine sample achieved the best tasting score (Popovic-Djordjevic et al. 2017).

This study aimed to determinate the content of 27 elements in the samples of vineyard soil, CF grapes and wines of the mother clone (standard) and its clones (Nos. 02, 010 and 012) by inductively coupled plasma - optical emission spectroscopy (ICP - OES). The overall aim was to estimate how clonal selection affected the elemental content of each single clone. Such a knowledge might be of particular importance for possible recommendation of any of these clones for introduction in viticultural practice, immediately after its commercialisation. According to the best of our knowledge, there are yet no any recognised CF clone developed in Serbia.

2. Material and methods

2.1. Experimental plot

The vineyard was planted at "Radmilovac" (Experimental field, Faculty of Agriculture, University of Belgrade) in Grocka wine growing region, with coordinates 44° 45′ N / 20° 35′ E, at an altitude of 153 m above the sea level. This locality situated in the area of Šumadija and Velika Morava (Serbia) has a moderate continental climate. The examined CF grape samples within this study were grown under the same conditions.

2.2. Chemicals

All chemicals (nitric acid, hydrochloric acid and hydrogen peroxide) were purchased for ICP-OES at analytical grade (trace metals analysis) from Sigma-Aldrich (Steinheim, Germany). Distilled and deionised water was used (Milli-Q Water System, Millipore Corporation, Bedford, MA).

2.3. ICP-OES analysis

2.3.1. Grapes

The grapes of Cabernet Franc mother clone (standard) and its clones (Nos. 02, 010 and 012) from the last phase of clonal selection were harvested at a full maturity (October 2015). These samples were firstly blended. Afterwards, 2 g of each sample were digested in a microwave digestion vessel system MBS-9 (CEM Innovators, Great Britain) using a mixture of nitric acid (10 ml) and hydrogen peroxide (3 ml) (EPA Methods 3051A, 2007). After cooling to room temperature, the solution was transferred into a clean volumetric flask and diluted to a final volume with ultra pure water.

Each clone and mother clone (standard) were represented by 50 vines planted in the random block design. All vines were grafted using a tongue grafting on the Kober 5BB rootstock. Planting distance was 3.0 m × 1.0 m (between rows and vines respectively), while the training system was a double asymmetric cordon. Pruning was done uniformly, leaving 20 buds per vine. There was no irrigation applied in a vineyard and all vines were grown under the same conditions (Popovic-Djordjevic et al. 2017).

2.3.2. Wines

Immediately after harvesting the grape samples were processed by microvinification technique as previously described (Pantelić et al. 2016). Fruit (50 kg) of each clone and the mother clone (standard) were harvested when berries reached total soluble solid of approximately 22 o Brix. Crushing was done manually using grape crusher/destemer with an addition of 100 mg/L potassium metabisulfite. Alcoholic fermentation was performed by *Saccharomyces cerevisiae* yeast (BDX) (0.2 g/kg) in 10 L glass vessels at temperature of 20-25 °C with the cap plunged twice a day. Following pressing, the obtained wine was transferred into glass balloons, sealed with an airlock that prevented the penetration of air into the space above the surface of the wine. Upon the completion of the fermentation, wines were racked from gross lees and cold stabilised. The content of free sulphur dioxide was adjusted to 20-25 mg/L (total SO_2 approximately 80 mg/L). Wines were then bottled in dark glass bottles with a cork closure and stored horizontally in a cellar at 10-12 °C with relative humidity of around 80%, until chemical analysis.

The elemental content of the wine samples was determined as followed. Firstly, the samples were diluted (1:5) with water containing 1% (v/v) nitric acid. The standards were prepared with 2.5% (v/v) ethanol and 1% (v/v) nitric acid, due to the same concentrations of ethanol and nitric acid, compared to the samples (Method OIV-MA-AS322-13, 2013).

2.3.3. Soil

Soil samples at two depths (0–55 and 55-102 cm) and several sites were collected from the vineyard at the experimental field "Radmilovac". Eutric Cambisols soil type, also called Gajnjača, belongs to the cambic class of soils, according to ex-Yugoslav classification system (Skoric et al. 1985). It is considered as the best one for grapevine production. This soil is well aerated through the whole solumn, thus enabling constant macro- to micro-pore ratio in the rhizosphere. Neutral reaction of soil was observed: pH slightly decreased with depth.

Prior to analysis, the soil samples were air-dried, sieved through stainless sieve and ground to a fine powder with a pestle in an agate mortar. Then, 1 g (dry weight) of each sample was digested with nitric acid, hydrogen peroxide and hydrochloric acid. After cooling, the samples were filtered and diluted to a final volume (EPA Methods 3050B, 1996). For each depth, the measurements were performed in triplicate. Finally, the results are expressed as the mean value of both depths with standard deviation.

2.3.4. Procedure

The content of 27 elements (Ag, Al, As, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sb, Sr, Tl, V and Zn) in all samples was determined by inductively-coupled plasma with optical emission spectrometer (ICP-OES) (SPECTROMETAR ICP-OES SPECTRO BLUE TI - SPECTRO Analytical Instruments GmbH, Germany). For calibration, a multi-element ICP standard (Roth Carl) and periodic table mix 1 ICP (Fluka) were used. The metals in soil standard reference material (ERA United States, 16,341 Table Mountain Parkway Golden, Colorado 80,403) were digested in triplicate and analysed to support quality assurance and control. Data of all measurements (obtained in triplicate) are expressed as the mean value with standard deviation.

2.3.5. Bioaccumulation factor

Grape/soil bioaccumulation factors (BF) were calculated as previously described (Milićević et al. 2017). The corresponding ratio was determined applying the following formula: BF = Cp/Cs, where Cp represents a concentration of the major or trace element in CF grapes, while Cs stands for the concentration of the same element in the soil sample.

2.3.6. Statistical analysis

The obtained experimental data were processed by appropriate methods using the package IBM SPSS Statistics 21. All values were processed using descriptive statistics indicators, while the statistical significance of the differences was estimated by ANOVA and Tukey test, at significance level of 5%. Relative dependence of the variables was measured by Pearson's correlation coefficient and tested by *t*-test, at the same level of significance (Hadživuković 1991).

Principal Component Analysis (PCA) and Hierarchical Cluster