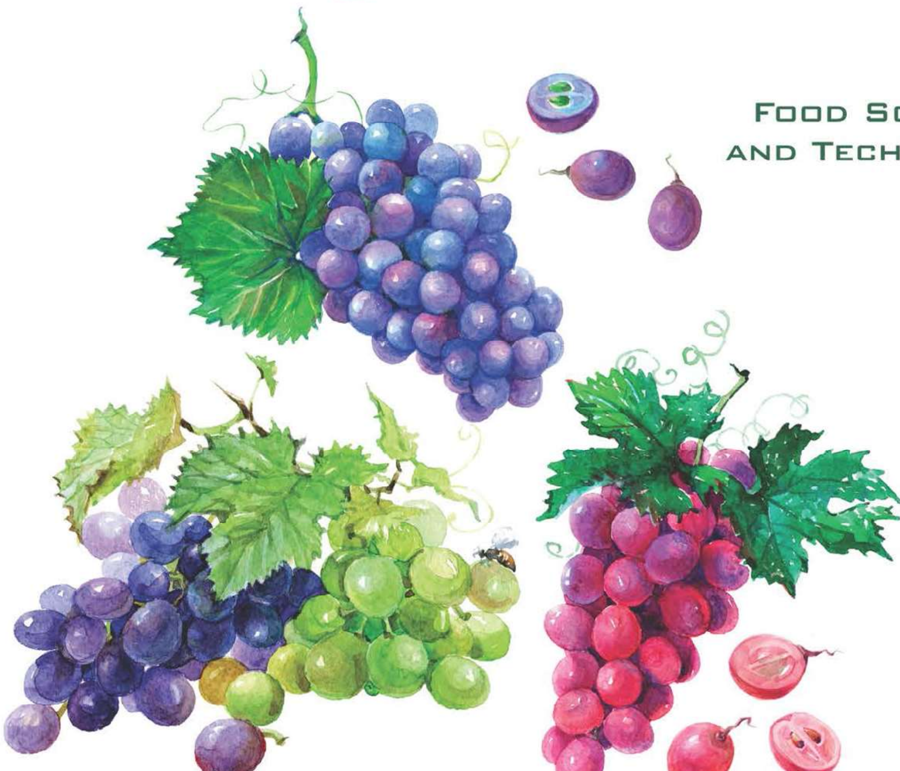
Grapevines at a Glance



Food Science and Technology

Josephine Estrada
Editor

NOVA

CONTENTS

In: Grapevines at a Glance
Editor: Josephine Estrada
ISBN: 978-1-53616-399-5

Chapter 2

CHARACTERIZATION AND CLONAL SELECTION OF SERBIAN AUTOCHTHONOUS VARIETY PROKUPAC (*VITIS VINIFERA* L.)

Milica M. Sredojević[1]***, Slavica R. Todić***[2]***, Nebojša R. Marković***[2]***, Dragana Č. Dabić Zagorac***[1] ***and Maja M. Natić***[3]

[1]Innovation center of the Faculty of Chemistry, Belgrade, Serbia
[2]Faculty of Agriculture-University of Belgrade, Serbia
[3]Faculty of Chemistry-University of Belgrade, Belgrade, Serbia

ABSTRACT

The first reported occurrence of *Vitis vinifera* in Balkan dates in the Neolithic period in the form of wild grape. Early traces of viticulture and winemaking in the territory of Serbia are vessels from the Iron Age (~ 400 BC) and the Bronze Age (~ 200 BC). Over the last few decades, interest in autochthonous varieties in Serbia is constantly increasing, especially for growing native varieties, such as Prokupac, Smederevka, Tamjanika and Bagrina. Prokupac is the most important Serbian autochthonous red wine variety (the first written records about growing Prokupac in Serbia date from 12th century), well adapted to the ecological conditions of its birthplace with corresponding phonological features. When compared with germplasm representing the classical eco-geographic grouping of grapevine cultivars, Prokupac is clustered within the *Convar pontica subconvar balcanica* taxon, supporting his indigenous origin. The main objective of this work is to present characterization of the Prokupac variety by means of ampelographic and molecular analysis, as well as grape and wine chemical characteristics. Long-term cultivation in diverse ecological conditions has caused the Prokupac to become a mixture of clones (genotypes). Therefore in order to preserve and improve autochthonous grapevine varieties in Serbia, work on variety clonal selection was initiated and till now, 12 clones of Prokupac were already identified and recognized. The most important features of these clones will be also presented.

Keywords: Prokupac, autochthonous variety, ampelographic characterization, clonal characterization, chemical characterization

1. Introduction

Serbia is located on the Balkan Peninsula and has a very long history of grape growing and winemaking. Grapevines have been grown on the territory of the present-day Serbia for more than a thousand years, as evidenced by the fossil remnants of grapevine seeds and the wine vessels that were discovered beside the Danube River, in Vinča, and in other archeological sites in Serbia [1]. These wine vessels are considered to be from the Bronze and Iron Ages [2]. It is thought that the greatest role in the development of viticulture in the region belonged to the ancient Romans, who not only planted grapevines and made wine, but also performed a classification, study and description of grapevine varieties, and have also written about their pests and diseases.

After becoming a part of the Ottoman Empire, Serbia's viticulture focused increasingly on the cultivation of table grapes, mainly through introduction of varieties from the Middle East. At the end of the 19th century, Serbian viticulture suffered the same fate as that of most Europe, namely the expansion of disease-causing agents from North America that caused a devastation of many vineyards. After a recovery period, particularly following the World War II, new vineyard areas were planted with varieties mainly introduced from France.

The assortment of grapevine varieties planted in Serbia has gradually been changing over time. These changes were caused by a number of factors, ranging from the introduction of new diseases to responses to changed social or geo-political factors (particularly following the two World Wars).

In the past, varieties grown in Serbia mainly were genotypes characteristic for the Balkan Peninsula and the Pannonia plains. Ecologically and geographically these varieties are classified as the group *Convarietas pontica*, *Subconvarietas balcanica*. Unfortunately, over time, quite a few of these varieties were neglected, abandoned in the production practice, and now only a few can be found in very old vineyards. First written documents with ampelographic descriptions of 25 varieties grown in Serbia in the past were authored by the archimandrite Prokopije Bolić in his 1816. book "Sovršeni vinodelac" (Perfect Winegrower).

The current assortment of varieties in Serbian vineyards is comprised of three groups of varieties: old domestic and domesticated wine and table grape varieties (1), introduced Western European varieties (2) and newly created domestic wine and table grape varieties (3) [3].

Old domestic (autochthonous) varieties originated in the Balkans, whereas the old domesticated (allochthonous) varieties were brought a long time ago from various geographical origins after which they have undergone a long process of natural selection and eventually have successfully adapted themselves to the Serbian environmental conditions. Uprooting old vineyards caused the loss of most of these varieties, although it is still possible to find some of them in the aged plantations.

In the last few decades, Serbia has seen a resurgence of interest in the traditional grape wine varieties such as Prokupac, Smederevka, Tamjanika, Začinak, Bagrina and several other minor varieties. Prokupac and Tamjanika are considered to be the oldest autochthonous Serbian grape varieties [4, 5]. These autochthonous and allochthonous varieties are a part of the biodiversity of the Balkans and also a part of the Serbian cultural heritage. Identification and preservation of these varieties require their detailed characterization and clonal selection in order to produce certified clonal planting material. Production of authentic wines made from autochthonous varieties can help Serbia find its place in the demanding and crowded world wine market.

Prokupac is currently the most economically important and the most widely planted among all the autochthonous varieties in Serbia. It is sometimes also found under its synonyms: Kameničarka, Rekovačka Crnka, Nikodimka, Rskavac, Prokupka and Niševka. Historically, and this is still the case today, largest vineyard area planted with Prokupac can be found in the Toplica and Župa wine regions (southern central Serbia). The name of this variety is linked to Prokuplje, a central town of the Toplica county. These southern areas have very favorable climatic conditions for this late ripening variety.

2. Biological and Technological Characteristics

Prokupac is a very vigorous and a high yielding grapevine variety. Budburst (beginning of the growing season, Figure 1a) occurs somewhat late, and grape maturity is also late (end of the 3^{rd} epoch of ripening). Given that Prokupac tends to be high yielding, spur pruning is recommended. Cluster thinning at the beginning of *véraison* and partial defoliation at fruit set are normally recommended as production techniques to significantly improve grape quality in this variety [6]. Well aerated, non-fertile and warm soil types suit Prokupac. Its dark blue berries are medium large (Figure 1b), round or slightly flattened, with a thick skin. Berry sugar concentration is medium to high (depending on the yield) and total acidity in must is high. Wines are ruby red in color, characteristically with red fruit aromas (sour cherry is dominant). Ageing in barrique helps the Prokupac wines to achieve fine tannin structure.

Prokupac is sensitive to downy mildew and moderately sensitive to powdery mildew. It is quite resilient towards *Botrytis*. Prokupac is moderately tolerant of winter frosts, and is not very sensitive to spring frosts due to the fact that budburst is relatively late.

(a) (b)

Figure 1. Serbian autochthonous variety Prokupac – (a) budburst and (b) mature bunch.

3. Ampelographic Description and Molecular Data

Ampelographic analysis is a method used for description of morphological features of grapevine varieties, and this is mainly performed using codes provided by the OIV "Primary descriptor priority list". Ampelographic description of Prokupac (Table 1) was performed using young shoots (OIV 001, 004), shoots (OIV 016), young leaves (OIV 051), mature leaves (OIV 067, 068, 070, 076, 079, 081-2, 084, 087), and berries (OIV 223, 225). This morphological description was performed on the non-clonal (population) Prokupac grapevines growing in the collection vineyard of the Experimental Station "Radmilovac", which belongs to the Faculty of Agriculture at the University of Belgrade. The reported description data (Table 1) were reprinted from the ampelographic varietal description database maintained in the Experimental Station "Radmilovac" (the printed version).

At this stage, the precise geographic origin and parentage of Prokupac have still not been determined.

During the inventory of Balkan grapevine samples [7] it was found that the sample for Prokupac, obtained from the Sremski Karlovci (Serbia) collection vineyard resulted in a distinct allelic profile catalogued as Prokupac in the Davis (USA) collection. However, these samples shared only 73% of alleles analyzed at 22 loci. In further investigation, it was confirmed that Prokupac from Serbia is different from Prokupac from the Vassal collection ("Domaine de Vassal" French Grape Germplasm Repository) [8]. In contrast, another Prokupac sample taken from Bosnia and Herzegovina was a genotype identical to Prokupac from the Davis collection and from the Vassal collection.

The parentage analysis revealed that Prokupac from the Sremski Karlovci collection vineyard might be a progeny of "true-to-type" Prokupac and Terrano (Ibid.). The same authors explained that this misnomer occurred as that the progenies of a particular parent variety were sometimes treated as if they were the same as the parent variety due to a misleading documentation or mislabeling [8]. Molecular investigations that aimed to examine the relationships among the traditional grape varieties grown in Serbia [4] showed that the Prokupac sample investigated in that study was a perfect match to the SSR profile of the Prokupac accession which is maintained in the DEU098 collection.

Table 1. Ampelographic description of Prokupac performed by the OIV "Primary descriptor priority list"

OIV Code N°	Descriptor	Notes
001	Young shoot: aperture of tip	5 (fully open)
004	Young shoot: density of prostrate hairs on tip	5-7 (medium to high)
016	Shoot: number of consecutive tendrils	1 (two or less)
051	Young leaf: color of upper side of blade (4th leaf)	2 (yellow)
067	Mature leaf: shape of blade	3 (pentagonale)
068	Mature leaf: number of lobes	2 (three)
070	Mature leaf: area of anthocyanin coloration of main veins on upper side of blade	5 (red beyond the 2^{nd} bifurcation)
076	Mature leaf: shape of teeth	3 (both sides convex)
079	Mature leaf: degree of opening/overlapping of petiole sinus	3 (open)
081	Mature leaf: petiole sinus base limited by veins	1 (none)
084	Mature leaf: density of prostrate hairs between main veins on lower side of blade	5 (medium)
087	Mature leaf: density of erect hairs on main veins on lower side of blade	7 (high)
223	Berry: shape	2 (globose)
225	Berry: color of skin	6 (blue black)

4. Clonal Selection

Clonal selection is considered as an important tool for grapevine genetic improvement [9]. *Vitis vinifera* L. varietal clones are selected to achieve improved grape quality attributes, more intense wine aroma and improved color, as well as for genetic resistance to pests and diseases [10].

Prokupac is a variety with a very heterogeneous population in Serbia. There are a high number of biotypes within this variety widely grown in all wine regions of Serbia – this indicates a long history of cultivation of this variety in Serbia. Long-term cultivation in variable environmental conditions across the country caused this variety to become a mixture of clones (different genotypes). Polyclonal origin and the accumulation of genetic mutations were understood to have caused a high variability of attributes within this variety [11].

Heterogeneity of a cultivated grapevine population can create numerous problems in vineyards and wine production, thus it is important to identify clones with desirable viticultural and winemaking properties. The main aims of clonal selection of Prokupac are geared towards identification of clones of more moderate vigor, smaller sized berries, earlier time of ripening, and improved anthocyanins concentration in berry skins and a phenolic structure in fruit that is more favorable for the production of high quality wines [10].

Clonal selection of Prokupac in Serbia commenced around 2000 [10, 12-15]. To this day, 25 potential clones (or "candidate clones") have been selected out of the population spread across various localities in Serbia. Out of these, 13 clones have now been recognized by the Serbian Ministry of Agriculture and have been added to the list of recognized varieties and clones of Serbia. The remaining potential clones are still undergoing investigations in the experimental plantations of agricultural departments within the University of Belgrade and the University of Novi Sad.

The Prokupac clones that have been recognized are: 40/5;40/8; 41/1; 41/3; 41/4; 41/6; 42/1; 42/2; 43/2; 43/4; 43/5; 43/6; 43/7.

Table 2. Mechanical properties of bunch and berry of Prokupac clones

Clone	Bunch length	Bunch width	Berry number per bunch	Bunch weight	Bunch structure		Seed number per berry	Seed weight	Berry weight	Berry structure			Sugar content %	Total acid content g/l	Glicoacidometric index
					% of stem	% of berry				% skin	% seed	% flash			
40/1	13,83	7,87	93,60	190,36	4,04	95,96	1,46	0,042	2,4	5,13	2,50	92,38	18,75	6,91	2,76
40/2	14,66	9,99	111,78	244,77	3,67	96,33	1,27	0,040	2,18	4,59	2,11	93,30	18,58	7,57	2,40
40/3	14,53	8,12	94,51	217,92	3,89	96,11	1,75	0,039	2,47	4,86	2,55	92,59	19,03	6,80	2,84
40/4	13,45	9,20	115,60	261,93	4,36	95,64	1,36	0,035	2,42	5,08	1,90	93,02	16,80	8,38	1,95
40/5	14,50	9,13	110,40	239,90	3,92	96,08	1,51	0,038	2,4	5,54	2,50	91,96	17,82	8,53	2,37
40/6	14,85	8,05	86,70	188,17	4,29	95,71	1,58	0,038	2	6,50	3,15	90,35	17,90	6,00	2,99
40/7	14,09	7,83	91,84	222,28	3,23	96,77	1,75	0,039	2,62	5,57	2,52	91,91	19,67	6,68	2,97
40/8	13,50	7,60	93,47	203,75	3,37	96,63	1,76	0,042	2,78	4,89	2,52	92,59	18,55	6,15	2,98
41/1	15,54	8,24	96,93	263,34	3,17	96,83	1,84	0,040	2,63	4,41	2,66	92,93	19,09	6,87	2,75
41/2	12,10	6,64	77,13	180,52	3,49	96,51	1,73	0,044	2,52	5,79	2,62	91,59	20,03	6,75	3,44
41/3	13,38	7,69	92,80	211,19	5,30	94,70	1,98	0,040	2,77	5,42	2,64	91,95	19,32	8,02	2,50
41/4	12,70	7,40	87,90	194,73	4,23	95,77	1,79	0,036	2,35	5,53	2,98	91,49	17,03	8,12	2,20
41/6	13,90	8,14	73,22	210,09	3,90	96,10	1,73	0,039	2,28	4,82	2,46	92,72	18,57	6,47	2,97
42/1	12,56	7,54	99,09	232,93	3,36	96,64	1,70	0,047	2,6	5,77	2,92	91,31	20,10	5,34	3,95
42/2	12,94	7,81	99,69	170,37	4,72	95,28	1,52	0,038	2,17	5,39	2,30	92,30	19,10	6,43	3,82
42/3	12,87	8,19	99,89	220,92	3,84	96,16	1,54	0,037	2,33	5,15	1,55	93,30	18,93	5,67	3,79
42/4	12,24	6,93	94,11	199,66	3,78	96,22	1,62	0,040	2,22	5,41	2,70	91,89	18,38	6,92	3,04
43/1	13,09	7,37	89,29	217,13	3,33	96,67	1,45	0,037	2,53	4,86	1,34	93,79	18,57	6,95	3,11
43/2	14,36	6,84	88,76	188,50	3,05	96,95	3,28	0,035	2,17	3,50	2,23	94,27	22,05	6,22	3,81
43/3	14,80	8,20	103,30	220,52	2,73	97,27	1,68	0,042	2,73	4,76	2,56	92,67	19,00	6,42	3,02
43/4	12,67	10,24	98,76	207,59	3,60	96,40	1,70	0,035	2,37	4,35	2,36	93,29	18,05	5,37	3,47
43/5	12,90	6,80	87,84	194,02	3,27	96,73	1,98	0,037	2,65	5,40	2,75	91,85	21,77	6,56	3,48
43/6	13,24	6,40	98,44	217,70	3,24	96,76	1,60	0,036	2,5	3,84	2,40	93,76	21,38	6,79	3,38
43/7	14,02	6,62	93,11	207,12	3,11	96,89	1,89	0,039	2,47	4,98	2,96	92,06	18,22	7,25	2,61
43/8	13,00	8,08	131,04	284,46	2,97	97,03	1,75	0,035	2,51	3,43	2,23	94,34	16,48	6,58	2,61
CV	6,69	12,44	12,38	12,67	16,16	0,62	21,30	7,83	8,28	14,27	16,91	1,04	7,29	12,37	17,77

During a three year trial period, all 25 recognized and potential clones have been investigated [11] in order to: examine the variability of morphological traits within this clonal collection (1); to calculate the correlation among the observed characteristics (2); to detect associations among the clones (3); to identify the most useful variables for discriminating among these clones (4); and finally to recommend the clones deemed suitable for further commercial utilization based on a number of favorable characteristics.

Results (Ibid.) indicate a considerable morphological and biological diversity among the investigated 25 clones. Morphological characteristics of berries and clusters (Table 2) showed a large degree of variability, as measured by the coefficients of variation (CV) which ranged 0.62 - 21.30%. The mean values of all observed traits have exhibited pronounced differences between the clones, indicating a high level of morphological variation [11]. This was additionally confirmed by the relatively large values of CV that were determined for a majority of the observed attributes (Table 2).

Based on all the examined traits and by applying a hierarchical cluster analysis (Ibid.), a dendrogram of phenotypic differences between the analyzed Prokupac clones was constructed [11]. Prokupac clones are related in many different ways, which explains the existence of numerous hierarchical levels. Cluster analysis has determined three clusters: clusters I, II and III, which included 7, 12 and 6 clones, respectively. This classification was mostly influenced by the bunch weight.

Principal Component Analysis (PCA) was further used to identify the most significant variables, and as a result five Principal Components (PCs) with Eigenvalues greater than 1 were isolated (Table 3) [11]. Results of the PCA suggested that 25 morphological and biological characteristics studied could be reduced to the three main characteristics: cluster size, cluster and berry structure, and berry size. The obtained information is entirely adequate considering the number of involved variables and the purpose of the study (Ibid.). The first PC determined represented 25.68% of the variability and was correlated with the number of berries and cluster weight. The second PC (24.80% of total variability) correlated with the cluster structure traits, and the third PC (12.88% of all variability) was correlated with berry weight. These three PCs reported in [11] can reduce the number of properties that need to be studied in the clonal selection of Prokupac. Those attributes are primarily related to the cluster and berry size and their structure. Ibid. also reported the results of the correlation analysis for the observed cluster and berry traits in the potential clones (Table 3). According to [16] this kind of result indicates a moderate genetic diversity between the clones and suggests evaluation of different morphological characteristics might be necessary for a meaningful clonal characterization.

Most of the Prokupac clones studied in [11] have been characterized by a moderate cluster size, favorable cluster and berry structure, with the exception of the clones 40/4, 41/1 and 43/8 which had large clusters. These particular clones, given those attributes, can be recommended for use in the production of wine distillates (Ibid).

Table 3. Eigenvalues-proportion of total variability and correlation between the original variables and the first five principal components for Prokupac clones

Variable	PC1	PC2	PC3	PC4	PC5
Bunch length	0,277	- 0,048	- 0,344	0,489	- 0,588
Bunch width	0,663	0,235	0,067	- 0,256	- 0,459
Berry number per bunch	0,796	- 0,116	- 0,093	- 0,202	0,015
Bunch weight	0,804	- 0,195	- 0,370	- 0,097	0,030
% of stem	0,010	0,825	0,424	0,058	0,120
% of berry	- 0,010	- 0,825	- 0,424	- 0,058	- 0,120
Seed number per berry	- 0,363	- 0,504	0,166	0,606	- 0,035
Seed weight	- 0,420	0,131	- 0,617	- 0,351	- 0,022
Berry weight	- 0,014	- 0,227	- 0,718	- 0,074	0,569
% of skin	- 0,449	0,768	- 0,179	- 0,213	- 0,053
% of seed	- 0,486	0,325	- 0,491	0,305	- 0,225
% of meet	0,544	- 0,711	0,345	0,027	0,137
Sugar content	- 0,635	- 0,500	0,067	0,170	0,086
Total acid content	0,448	0,491	- 0,121	0,512	0,397
Glicoacidometric index	- 0,640	- 0,487	0,318	- 0,405	- 0,131
Eigenvalue	3.852	3.720	1.931	1.452	1.166
% Variance	25.68	24.80	12.88	9.68	7.78
% Cumulative	25.68	50.48	63.36	73.04	80.82

5. Chemical Composition of Prokupac Grape and Wine

In most recent studies which included some international and autochthonous varieties from Serbia, phenolic profile and antioxidant potential of Prokupac grape and wine was studied. Available literature provides data about the abundance of phenolic compounds in whole berries, as well as in different parts: skins, seeds and pulp. Also, there are some literature data about elemental composition. Chemical traits are presented in the following section of the Chapter, with a special emphasis on the phenolic compounds.

5.1. Composition of the Grape, Skin, Seed and Pulp

Composition of the whole Prokupac grape berries was investigated for the samples collected in a vineyard located in South Serbia [17]. Authors reported on the antioxidant properties, phenolic profile, total phenolic (TPC), total flavonoid (TFC), and total anthocyanin (TAC) content, as well as individual polyphenols. TPC was 156.28 mg gallic acid equivalents per 100 g fresh weight of grape (mg GAE/100 g), TFC was 105.80 mg CE/100 g, while TAC amounted 88.80 mg malvidin-3-O-glucoside/100 g. Result for TPC obtained herein was in the range of values determined for different table and wine grapes from various countries (63 - 480 mg GAE/100 g).

Also, a total of 10 anthocyanins were identified in grape extract due to their UV-Vis spectra by using HPLC-DAD, and the findings were that Prokupac was particularly rich in malvidin-3-O-glucoside [17]. Among *p*-coumaroylmonoglucosides, the most abundant were peonidin-3-*p*-coumaroylmonoglucoside and malvidin-3-*p*-coumaroylmonoglucoside. Besides, two flavan-3-ols (catechin (1.12 mg/kg) and epicatechin (0.92 mg/kg)) and four hydroxycinnamic acids were identified and quantified (*t*-caftaric acid (0.47 mg/kg), *c*-coutaric acid (0.22 mg/kg), *t*-coutaric acid (0.21 mg/kg), and *t*-fertaric acid (0.11 mg/kg).

One of the first reports on chemical composition of the seeds was phenolic composition of the seeds extracts screened by the means of HPLC/PDA/ESI/MS [18]. As a result of this investigation, 22 phenolic compounds were quantified and 7 more compounds were present in traces, all of them classified into flavan-3-ol monomers, proanthocyanidins, flavonols, hydroxycinnamic acid, and hydroxybenzoic acid derivatives. Also, later studies were conducted and 20 phenolic compounds were identified and quantified in seeds using UHPLC-DAD MS/MS [19]. Flavan-3-ols were the dominant polyphenols, with a catechin being the most abundant (1111.66 mg/kg dry weight), followed by gallocatechin gallate (788.80 mg/kg dry weight). In the most recent study, 4 structurally distinct groups of flavan-3-ols were identified, being 3 monomeric flavan-3-ols, 2 procyanidin isomers A type, 17 procyanidin isomers B type, and 11 procyanidin gallate isomers [20]. Identification was achieved using UHPLC-Orbitrap MS and the quantities of individual compounds were expressed as equivalents of catechin. The results obtained in this paper indicated that relative amounts of the oligomers of procyanidin (dimers to tetramers) were higher when compared with the monomers, with procyanidin isomers B type being the most abundant (approximately 50%).

Besides flavan-3-ols, literature survey showed grape seed extracts to be abundant in phenolic acids, with ellagic acid and gallic acid being prevalent [18 - 20]. Also, in comparison to some international grapevine varieties, higher contents of these two acids were found in Prokupac grape seeds [19]. These findings were interesting as identification of ellagic acid and ellagic acid glycoside in Prokupac seeds was not expected, as the existence was believed to be unique for muscadine grapes (*Vitis rotundifolia*) [21]. Among flavonols, quercetin was the most abundant [19, 20]. Finally, seeds of this autochthonous variety were characterized with higher contents of some flavan-3-ols (catechin, epigallocatechin gallate, epicatechin, gallocatechin gallate, and catechin gallate) and naringin, in comparison to the samples obtained from international grapevine varieties that were also investigated in the work [19]. Results also pointed to the higher RSA and TPC values (967.90 µmol trolox eqivalents per g of dried seeds (µmol TE/g DW) and 101.25 mg GAE/g DW, respectively) of

Prokupac seeds in comparison to the other varieties. Composition of bioavailable elements (Al, Cd, Cr, Cu, Fe, K, Ni, Pb, and Zn) determined using inductively coupled plasma optical emission spectroscopy (ICP-OES), was also published [22]. As expected, potassium was the most abundant element in seeds (1108 μg/g). The rest of the elements were detected in significantly lower concentrations.

Composition of the skins was reported recently and results showed the presence of phenolic acids and flavonols, representing 90.3% of the total polyphenols in grape skin extract (24.4% and 65.9%, respectively) [20]. These results are generally in line with those previously published by Pantelić et al. [19], when quantification of phenolics in Prokupac grape skins revealed 17 compounds, of which 9 were phenolic acids and 5 were flavonols. According to Pešić et al. [20], ellagic acid was the major phenolic acid in the extract of grape skin (635 μg/L), while previously results pointed to prevalence of gentisic and gallic acids (7.15 mg/kg and 3.90 mg/kg, respectively) [19]. Among flavonols, quercetin and isorhamnetin, together with their glycosides, were detected in significant amounts [19, 20]. The presence of one flavan-3-ol (epigallocatechin) in Prokupac grape skin was reported in [19], while in [20] this class of phenolics was not found. The presence of resveratrol was also observed in the skins (13.42 mg/kg frozen weight) [19]. Of all phenolic compounds identified in Prokupac grape skin, anthocyanins amounted approximately 6% [20]. Such a small proportions of the anthocyanins were expected as Prokupac was already declared as a grape variety that contains moderate amounts of anthocyanins in comparison to some international varieties (Cardinal, Ribier, Muscat Hamburg, Merlot, and Cabernet Sauvignon) and autochthonous variety Vranac [17].

According to presented data malvidin-3-*O*-glucoside was the most abundant anthocyanin in grape skin extracts, followed by peonidin- 3-O-glucoside [19, 20]. Interestingly, the presence of malvidin 3,5-*O*-dihexoside in the berry skin of Prokupac was confirmed, which is untypical for *Vitis vinifera* Linneo species [19].

As for total phenolic content and radical scavenging activity (12.32 mg GAE/g frozen weight (FW) and 132.59 μmol TE/g FW, respectively), skin of Prokupac variety was characterised with higher values when compared to the rest of 12 grape samples investigated in [19]. Different manipulation practices in vineyards are frequently used for the improvement of grape quality, which is also documented in scientific papers [6, 23 - 25]. As reported in [6], early defoliation increased contents of total phenolics and total anthocyanins in the skins of Prokupac variety (TPC ranged from 518.13 to 632.4 mg GAE/L, while TAC ranged from 5.61 to 6.35 mg/g FW). When it comes to TPC and RSA values, results indicate that early defoliation had more positive effect on Prokupac than on Cabernet Sauvignon.

Besides phenolic compounds, bioavailable elements (Al, Cd, Cr, Cu, Fe, K, Ni, Pb, and Zn) were isolated from Prokupac berry skin [22]. In comparison to international varieties investigated in this study (Riesling Rain, Burgundac, Cabernet Sauvignon, Riesling Italian, Cebarnet Franc, and Merlot), a skin of Prokupac grape had the highest concentration of Ni (52 μg/g). Bioaccumulation factors (BF) of all the elements were calculated, and based on the results the skin of Prokupac grapevine was suggested to be a plant accumulator of Ni from the soil.

Grape pulp was poorly studied and investigations done so far indicated that the content of chemical compounds in this tissue is significantly lower when compared to skins and seeds [19, 26]. Yet, results pointed to hydroxybenzoic acids as the most prevalent phenolic compounds in Prokupac grape pulps [19]. Besides phenolic acids, low contents of flavan-3-ols were also determined in the pulp sample. Interestingly, rutin, although found in the pulps of all examined international varieties, was not found in the pulp of Prokupac.

5.2. Composition of the Wine

Phenolic profile of young monovarietal Prokupac wine from the Central Serbia was recently investigated and compared with the wines obtained from some red and white international grapevine varieties [27]. Quantification of phenolics, using ultra-high performance liquid chromatography (UHPLC) coupled with a diode array detector and a triple-quadrupole mass spectrometer, revealed the presence of 22 compounds, and Prokupac was the only sample in which all compounds were identified. Phenolic acids were the most abundant class of phenolic compounds and the highest concentrations of hydroxybenzoic acids were found in Prokupac (total 40.63 mg/L). Prokupac wine stood out with the highest TPC and RSA, as well as with unique composition of the individual polyphenols. This was confirmed by principal component analysis, where clustering of red and white wines was achieved, with the Prokupac being the outlier. In comparison to the other analysed wines, several times higher contents of ellagic acid, caffeic acid, catechin, epicatechin, quercetin 3-O-galactoside, and myricetin was found in Prokupac wine, but also, it was the only one sample containing naringin, naringenin and resveratrol. On the contrary, when the influence of winemaking techniques and cultivars on the resveratrol content was investigated, the lowest total resveratrol content (*trans*- and *cis*-resveratrol) was found for wine obtained from this autochthonous cultivar [28]. The values obtained for resveratrol in Prokupac wine were in the range from 0.35 to 0.78 mg/L, while in other analysed samples it ranged from 1.12 mg/L (in Pinot Noir) to 4.85 mg/L (in Merlot).

Another investigation done on the Prokupac wine sample revealed a total of 15 identified and quantificated phenolic compounds by using HPLC-DAD, showing the presence of 5 anthocyanins, mainly malvidin, delphinidin, and peonidin monoglucosides, but also some acetyl and *p*-coumaroyl derivatives, and 10 non-anthocyanins [29]. As expected, the major anthocyanin in investigated red wine sample was malvidin-3-*O*-glucoside and it accounted 62.67% of total anthocyanins present in Prokupac wine, while the derivatives of cyanidin were not found. A prevalence of malvidin-3-*O*-glucoside in Prokupac wine was confirmed in two more studies [10, 30].

Based on spectrophotometric assays, TPC and RSA were reported for Prokupac wine, and markedly higher values were obtained in comparison with all international wines included in this research (2.60 g GAE/L and 15.06 mmol TE/L, respectively) [27]. Similar results for total phenolic content in Prokupac wine (354.81 mg GAE/100 mL) were obtained in [29], while according to Atanacković et al. [28], the opposite was shown, and lower TPC values were determined for Prokupac wine (from 544.37 to 1159.37 mg GAE/L) in comparison to the international grapevine varieties studied (Merlot, Cabernet Sauvignon and Pinot noir).

Also, chemical evaluation of wines obtained from 13 different clones of the autochthonous grape variety Prokupac discovered generally low values of total phenolic content in all investigated samples (range from 33.0 to 114.5 mg GAE/100 mL) [10]. In Prokupac wine samples produced from different clones, the contents of total anthocyanins and proanthocyanidins were determined as well. The values of total proanthocyanidins ranged from 33.0 mg GAE/100 mL to 114.5 mg GAE/100 mL, while the percentage content of anthocyanins was between 0.006% and 0.015% (expressed as cyaniding 3-glucoside chloride). Statistically significant differences in Prokupac wine quality between the clones were observed. Wines produced from clones 43/5 and 43/4 were of the highest quality, in terms of total phenolics, total anthocyanins and total proanthocyanidins.

Total anthocyanin content and total proanthocyanidins in Prokupac wine were also determined in [29] and the values wer 14.6 mg malvidin-3-glucoside/100 mL and 174.8 mg catechins/100 mL, respectively. According to results reported in [31] the contents of total phenolics, total flavonoids, and total anthocyanins, and antioxidant activity (EC_{50}) in Prokupac red wine were 343.4 mg GAE/L, 130.5 mg CTE/L, 60.6 mg/L, and 0.083 mg/mL, respectively. Phenolic profiles of Prokupac wine and radical scavenging activity of different fractions (aqueous and organic) were assessed applying liquid–liquid extraction [30]. Identified phenolic compounds were classified as anthocyanins, flavonols, flavan-3-ols, and phenolic acids. According to the results obtained using HPLC and LCMS, fractions differ in the terms of phenolic abundance. Anthocyanins were the main componenets in the aqueous residue, while non-anthocyanin phenolics dominated in EtOAc fractions (at pH 2.0 dominated hydroxycinnamic acids and quercetin 3-O-glucuronide, and at pH 7.0 fraction was characterised by the presence of catechins, hydroxybenzoic acids, and quercetin). Taking account all fractions, total phenolic contents ranged from 48.22 to 289.12 mg GAE/g dry fraction. The radical scavenging activities of the fraction differed significantly and the IC_{50} values in range from 3.47 µg/mL to 138.58 µg/mL.

Prokupac wine proved to be very suitable for coupling with other autochthonous and international red wines (e.g., Vranac and Gamay) [32]. This indigenous variety is often compared to some French vine varieties (Cabernet Sauvignon, Pinot Noir, and Gamey). For example, investigation [33] showed no significant differences in the antioxidant and antibacterial activities between the wines from Cabernet Sauvignon and Pinot Noir and the indigenous varieties (Vranac and Prokupac) were observed. Some papers indicated improved chemical characteristics, such as increased phenolic contents and antioxidant capacity, of beer and complex food matrix (meat- and cereal-based products) produced by the addition of the Prokupac grape [20, 34].

Finally, research on grapevine leaves that was conducted on numerous varieties, including several autochthonous such as Prokupac, Plovdina, and Smederevka, draw attention to the potency of grapevine leaves extracts as a good source of natural antioxidants. Due to high contents of phenolic compounds and especially due to abundance of ellagic acid, grapevine leaves can be used in traditional recipes and as supplements in food and medicines [35].

CONCLUSION

Thanks to the long history of cultivation of grapevines in Serbia under its favorable growing conditions, the country is rich in grapevine germplasm. The work on identification, origin and genetic relationships in old, traditional, varieties is important for the preservation of the grapevine gene fund in Serbia, and also for the development of the wine and viticulture industries. Molecular information for traditional varieties of Serbia is still not fully available. Further genetic research will be necessary to fully characterize the germplasm of Serbian grape varieties and to understand the genetic linkage between these varieties and the overall diversity of indigenous varieties of the region. This will lead to better utilization of these genetic resources for future grapevine selection and hybridization work.

Given the vital role autochthonous varieties and particularly Prokupac among them have in the recognition of Serbia as a wine country, detailed ampelographic, genetic and chemical characterizations have the highest priority for the Serbian wine industry.

Thirteen newly-identified clones of Prokupac have gained the official recognition thus far. The ongoing clonal selection should focus on first identifying, and then planting foundational vineyards, with new clones that have traits which will significantly improve the variety's potential for the production of fine wines.

References

[1] Ivanišević, D., Jakšić, D. & Korać, N. (2015). *Vinogradarski atlas. Popis poljoprivrede 2012*, Poljoprivreda u Republici Srbiji. Beograd: Republički zavod za statistiku. [*Vineyard Atlas. Census of Agriculture 2012*, Agriculture in the Republic of Serbia]

[2] Lazić S., Briza, K., Burić, D., Jazić, L. j., Zorzić, M., Kovač, V., Užić, N., Ranković, N., Zvekić, S., Karanović, V. & Dražić, S. (1976). *1700 godina vinogradarstva i vinarstva Fruške gore*. Novi Sad: Privredna komora Vojvodine. Grupacija za proizvodnju voća, grožđa i vina. [*1700 years of Fruska Gora viticulture and winemaking.* Novi Sad: Chamber of Commerce of Vojvodina. Fruit, grape and wine production group.]

[3] Korać, N., Cindric, P., Papric, D. j., Kuljancic, I., Medić, M., Ivanišević, D. & Božović, P. (2007). "Domestic and old adopted grapevine cultivars in Fruska Gora wine area region." *Savremena poljorivreda*, *56*, 248–255. [*Contemporary agriculture*]

[4] Beslic, Z., Todic, S., Korac, N., Lorenzi, S., Emanuelli, F. & Grando, M. S. (2012). "Genetic characterization and relationships of traditional grape cultivars from Serbia." *Vitis*, *51*, 183–189.

[5] Pivac, T. (2012). *Vinski turizam Vojvodine*, *Monografija*. Novi Sad: Univerzitet u Novom Sadu, Prirodno – matematički fakultet. [*Vojvodina Wine Tourism, Monograph*]

[6] Beslic, Z., Todic, S. & Matijasevic, S. (2013). "Effect of Timing of Basal Leaf Removal on Yield Components and Grape Quality of Grapevine cvs Cabernet Sauvignon and Prokupac (*Vitis vinifera* L.)." *Bulgarian Journal of Agricultural Science*, *19*, 96–102.

[7] Štajner N., Tomić, L., Ivanišević, D., Korać, N., Cvetković-Jovanović, T., Beleski, K., Angelova, E., Maraš, V. & Javornik, B. (2014). "Microsatellite inferred genetic diversity and structure of Western Balkan grapevines (*Vitis vinifera* L.)." *Tree Genetics & Genomes*, *10*, 127–140.

[8] Štajner, N., Tomić, L., Progar, V., Pokorn, T., Lacombe, T., Laucou, V., Boursiquot, J. M., Javornik, B. & Bacilieri, R. (2015). "Genetic clustering and parentage analysis of Western Balkan grapevines (*Vitis vinifera* L.)." *Vitis*, *54*, 67–72.

[9] Rakonjac, V., Korac, N., Todic, S., Medic, M., Bešlic, Z., Kuljancic, I., Ivaniševic, D. & Popov, M. (2014). "Genetic diversity of a Serbian grapevine germplasm collection based on morphoagronomic characteristics." *Genetika*, *46*, 719–730.

[10] Živković, J., Šavikin, K., Zdunić, G., Gođevac, D., Marković, N., Pržić, Z. & Menković, N. (2016). "Influence of bunch morphology on quality of wines produced from clones of grape variety Prokupac." *Journal of the Serbian Chemical Society*, *81*, 883–895.

[11] Marković, N., Pržić, Z., Rakonjac, V., Todić, S., Ranković-Vasić, Z., Matijašević, S. & Bešlić, Z. (2017). "Ampelographic characterization of *Vitis* cv "Prokupac" clones by multivariate analysis." *Romanian Biotechnological Letters*, *22*, 12868–12875.

[12] Marković, N., Todić, S., Jović, S., Bešlić, Z. & Petrović, A. (2008). "Biological and technological properties of cv. Prokupac clones."

Paper presented at the III Serbian congress of fruit and grape growers, Serbia, Belgrade.

[13] Sivcev, B., Rankovic-Vasic, Z. & Radovanovic, D. (2011). "Clone selection of autochthonous and introduced varieties in the old grapevine planted areas of south eastern and eastern Serbia and preliminary check of their health status." *Genetika*, *43*, 465–475.

[14] Marković, N., Atanacković, Z. & Ranković-Vasić, Z. (2013). "Uvometric and technological characteristics of Prokupac clones 42/1, 43/2 and 43/6." Paper presented at International *Symposium for Agriculture and Food-IV Macedonian Symposium for Viticulture and Wine Production*, FNRY Macedonia, Skopje, December 12–14.

[15] Marković, N. & Pržić, Z. (2018). "Clonal selection of prokupac grape variety in Serbia-current results." Annals of the University of Craiova - Agriculture, *Montanology*, *Cadastre Series*, *48*, 146–151.

[16] Reim, S., Profta, A., Heinz, S. & Hofer, M. (2012). "Diversity of the European indigenous wild apple *Malus sylvestris* (L.) Mill. in the East Ore Mountains (Osterzgebirge), Germany: I. Morphological characterization." *Genetic Resources and Crop Evolution*, *59*, 1101–1114.

[17] Mitić, M., Souquet, J. M., Obradović, M. & Mitić, S. (2012). "Phytochemical profiles and antioxidant activities of Serbian table and wine grapes." *Food Science and Biotechnology*, *21*, 1619–1626.

[18] Gođevac, D., Tešević, V., Veličković, M., Vujisić, L. j., Vajs, V. & Milosavljević, S. (2010). "Polyphenolic compounds in seeds from some grape cultivars grown in Serbia." *Journal of the Serbian Chemical Society*, *75*, 1641–1652.

[19] Pantelić, M., Dabić Zagorac, D., Davidović, S., Todić, S., Bešlić, Z., Gašić, U., Tešić, Ž. & Natić, M. (2016). "Identification and quantification of phenolic compounds in berry skin, pulp, and seeds in 13 grapevine varieties grown in Serbia." *Food Chemistry*, *211*, 243–252.

[20] Pešić, M., Milinčić, D., Kostić, A., Stanisavljević, N., Vukotić, G., Kojić, M., Gašić, U., Barać, M., Stanojević, S., Popović, D., Banjac, N. & Tešić, Ž. (2019). "*In vitro* digestion of meat- and cereal-based

food matrix enriched with grape extracts: how are polyphenol composition, bioaccessibility and antioxidant activity affected?" *Food Chemistry*, *284*, 28–44.

[21] Sandhu, A. & Gu, L. (2010). "Antioxidant capacity, phenolic content, and profiling of phenolic compounds in the seeds, skin, and pulp of *Vitis rotundifolia* (Muscadine grapes) as determined by HPLC-DAD-ESI-MSn." *Journal of Agricultural and Food Chemistry*, *58*, 4681−4692.

[22] Milićević, T., Relić, D., Škrivanj, S., Tešić, Ž. & Popović, A. (2017). "Assessment of major and trace element bioavailability in vineyard soil applying different single extraction procedures and pseudo-total digestion." *Chemosphere*, *171*, 284–293.

[23] Bešlić, Z., Pantelić, M., Dabić, D., Todić, S., Natić, M. & Tešić, Ž. (2015). "Effect of vineyard floor management on water regime, growth response, yield and grape quality of Cabernet Sauvignon." *Scientia Horticulturae*, *197*, 650–656.

[24] Rodríguez-Montealegre, R., Romero-Peces, R., Chacón-Vozmediano, J. L., Martínez-Gascueña, J. & García-Romero, E. (2006). "Phenolic compounds in skins and seeds of ten grape Vitis vinifera varieties grown in a warm climate." *Journal of Food Composition and Analysis*, *19*, 687–693.

[25] Rusjan, D., Veberič, R. & Mikulič-Petkovšek, M. (2012). "The response of phenolic compounds in grapes of the variety 'Chardonnay' (*Vitis vinifera* L.) to the infection by phytoplasma Bois noir." *European Journal of Plant Pathology*, *133*, 965–974.

[26] Pantelić, M., Dabić Zagorac, D., Natić, M., Gašić, U., Jović, S., Vujović, D. & Popović-Djordjević, J. (2016). "Impact of clonal variability on phenolics and radical scavenging activity of grapes and wines: A study on the recently developed Merlot and Cabernet Franc clones (*Vitis vinifera* L.)." *PLoS One*, *11*, e0163823. Accessed October 12, 2016. doi: 10.1371/journal.pone.0163823.

[27] Pantelić, M., Dabić Zagorac, D., Gašić, U., Jović, S., Bešlić, Z., Todić, S. & Natić, M. (2018). "Phenolic profiles of Serbian autochthonous variety 'Prokupac' and monovarietal international wines from the Central Serbia wine region." *Natural Product Research*, *32*, 2356–2359.

[28] Atanacković, M., Petrović, A., Jović, S., Gojković-Bukarica, L. j., Bursać, M. & Cvejić, J. (2012). "Influence of winemaking techniques on the resveratrol content, total phenolic content and antioxidant potential of red wines." *Food Chemistry*, *131*, 513–518.

[29] Mihailovic-Stanojevic, N., Savikin, K., Zivkovic, J., Zdunic, G., Miloradovic, Z., Ivanov, M. & Karanovic, D. (2016). "Moderate consumption of alcohol-free red wine provide more beneficial effects on systemic haemodynamics, lipid profile and oxidative stress in spontaneously hypertensive rats than red wine." *Journal of Functional Foods*, *26*, 719–730.

[30] Menković, N., Živković, J., Šavikin, K., Gođevac, D. & Zdunić, G. (2014). "Phenolic composition and free radical scavenging activity of wine produced from the Serbian autochthonous grape variety Prokupac–A model approach." *Journal of the Serbian Chemical Society*, *79*, 11–24.

[31] Lakićević, S., Popović Djordjević, J., Pejin, B., Djordjević, A., Matijašević, S. & Lazić, M. (2018). "An insight into chemical composition and bioactivity of 'Prokupac' red wine." *Natural Product Research*, *16*, 1–5.

[32] Radovanović, B., Radavanović, A. & Tomić, V. (2012). "Relations between the phenolic composition and free radical scavenging, and antibacterial activities of red wines from different cultivars of *Vitis vinifera* L." *International Journal of Food Properties*, *15*, 725–735.

[33] Radovanović, A., Radovanović, B. & Jovančićević, B. (2009). "Free radical scavenging and antibacterial activities of southern Serbian red wines." *Food Chemistry*, *117*, 326–331.

[34] Veljovic, M., Despotovic, S., Stojanovic, M., Pecic, S., Vukosavljevic, P., Belovic, M. & Leskosek-Cukalovic, I. (2015). "The fermentation kinetics and physicochemical properties of special beer with addition of Prokupac grape variety." *Chemical Industry and Chemical Engineering Quarterly*, *21*, 391−397.

[35] Pantelić, M., Dabić Zagorac, D., Ćirić, I., Pergal, M., Relić, D., Todić, S. & Natić, M. (2017). "Phenolic profiles, antioxidant activity and minerals in leaves of different grapevine varieties grown in Serbia." *Journal of Food Composition and Analysis*, *62*, 76-83.