

TECHNOLOGICAL CHARACTERISTICS OF INTERSPECIFIC HYBRIDS BRONNER, MUSCARIS AND MORAVA IN BANJA LUKA REGION

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ABSTRACT

Interspecific hybrids are increasingly finding their place in the assortment structure of modern viticulture, thanks to their notable resistance to diseases and adaptability to different climatic conditions. The aim of the research was to determine and compare the basic ampelographic and technological characteristics of the interspecific hybrids - Bronner, Muscaris and Morava with the conventional white wine variety Pinot Blanc, grown in the Banja Luka region (Bosnia and Herzegovina). The variety Muscaris stood out compared to the other analyzed varieties on the basis of the largest grape cluster weight (244.99 g), the weight of 100 grape berries (173.06 g), the weight of 100 grape skins (17.54 g) and the weight of seeds in 100 grape berries (11.50 g). This variety also had the highest sugar content in grape juice (27.63 °Brix), followed by the Bronner variety (24.67 °Brix), which, according to the OIV categorization, ranks them among varieties with a very high sugar content. The Morava and Pinot Blanc varieties had a relatively similar sugar content (22.02 and 21.77 °Brix, respectively). The content of total polyphenols in the skins of Bronner variety grapes was statistically significantly higher (1876.73 mg/kg) compared to the other analyzed varieties, while a similar situation was observed in the seeds of the Pinot Blanc variety (2913.68 mg/kg). Based on the results, it was concluded that the variety Muscaris showed the best qualitative characteristics.

Key words: Bronner, grape, interspecific variety, Morava, Muscaris, quality.

INTRODUCTION

The grapevine (*Vitis vinifera* L.) is one of the oldest and most widespread agricultural crops. The total worldwide area under grapevines intended for all purposes (production of wine and juices, table grape and raisins, as well as, young vines that are not yet at reproductive age) was 7.3 mha in 2020 (OIV, 2021).

The existing genetic diversity of the grapevine has largely been determined by the historical development of viticulture and different methods of grapevine cultivation (This et al., 2006). The history of European viticulture can be divided into three periods (Gessler et al., 2011). The first period (before 1845) is characterized by the absence of major phytosanitary issues. Then came the half-century period of facing and searching for solutions to the problems caused by the appearance of new diseases and pests: downy mildew and phylloxera. The last period, which continues to this day, was marked by the application of chemical protection in viticulture. With the appearance of the aforementioned diseases, a new chapter begins in the grapevine growing and breeding. According to Alleweldt (1997), French scientists then formulated two breeding goals: the development of rootstocks resistant or tolerant to phylloxera and the development of interspecific hybrids that would represent a combination of the quality of *Vitis vinifera* grapes with disease resistance of American species of the genus *Vitis*. The result of the above mentioned program was the creation of hybrids called "direct

producers" (Alleweldt & Possingham, 1988), and French-American hybrids, which remained in France until the second half of the 20th century (Reynolds, 2015). The spreading of the above-mentioned interspecific hybrids in France was gradually stopped, not only because of the poor wine quality, but also because of the legal regulations related to the cultivation prohibition (Crowley, 1993). In addition, French-American hybrids later remained part of breeding programs in other countries: Germany, Hungary, Russia, Ukraine, Moldova, etc., as the resistance gene donors (Töpfer et al., 2011; Karlagić Kontić, 2014). Resistance to the most significant diseases and low temperatures of certain American and East Asian species of the genus *Vitis* (*Vitis labrusca*, *Vitis riparia*, *Vitis rupestris*, *Vitis amurensis* etc.), multiple interspecific hybridization and characterization of different generations of interspecific hybrids have been the subject of work and study by many world researchers - breeders in the past and present (Koleda, 1975; Bouquet 1986; Eibach, 1994; Staud, 1997; Lu et al., 2000; Kozma, 2000; Pavloušek, 2007; Hajdu, 2015; Wang et al., 2020). Research conducted in the Western Balkans countries also made a great contribution in the mentioned segment (Cindrić & Kovač, 1988; Avramov, 1991; Žunić et al., 2002; Korać et al., 2005; Nikolić, 2006; Jovanović-Cvetković & Mijatović, 2007; Ivanišević et al., 2012; Radojević et al., 2012 ; Karlogan Kontić et al., 2016).

Environmental awareness has led to significant changes in the area of grape breeding, production methods and assortment selection. Breeding programs are aimed at creating varieties adaptable to the influence of biotic and abiotic factors, with high fruit quality and ripening period in accordance with market requirements. Resistance to fungal diseases and low temperatures is the primary goal of many breeding programs, thereby expanding the limits of grape production (Reisch et al., 2012). The result of the above mentioned programs is the creation of a respectable number of disease-resistant varieties in Europe and North America, applicable in conventional and sustainable grape production (Pedneault & Provost, 2016).

With their characteristics, interspecific hybrids meet the objectives of the mentioned breeding programs in many ways and they are part of the production assortment in countries dedicated to organic grape production (Germany, Italy, Hungary, etc.), in countries with cold climates (Lu & Liu, 2015; Diez-Zamudioetal et al., 2021; Kuang et al., 2022) and in countries with hot and dry conditions (Camargo et al., 2014; Volynkin et al., 2021). The positive economic, ecological and health-safe effects of grape and wine production from interspecific hybrids are indisputable, due to the reduction in fungicide usage, as much as 73-82 % (Jackson, 2014).

In Bosnia and Herzegovina (BiH), interspecific hybrids occupy insignificant areas, but there is a noticeable tendency of a slight increase in certain microregions. Mijatović & Vuksanović (1987) recommended the cultivation of the mentioned hybrids for experimental purposes by evaluating the pedoclimatic conditions of the northern parts of Bosnia and Herzegovina. In the last decade, the results of research of the agrobiological and technological characteristics of certain interspecific hybrids grown in the northern Bosnian (Kozara region) area are also available (Jovanović-Cvetković & Mijatović, 2009; Mijatović et al., 2015; Jovanović-Cvetković et al., 2017). The knowledge that certain interspecific hybrids are grown in these areas, whose characteristics have not been analyzed so far, was the reason for the research of some interspecific hybrids grown in the ecological conditions of the Banja Luka region (northwestern part of Bosnia and Herzegovina), in order to determine their ampelographic and technological characteristics.

MATERIAL AND METHODS

The research was conducted in 2021. The subjects of the research were three white wine interspecific varieties: Bronner, Muscaris and Morava and the conventional white wine variety Pinot Blanc (the standard variety in the experiment), grown in the Banja Luka region. Basic data on the origin of the analyzed varieties are given in Table 1.

Table 1. Basic data on the origin of the analyzed interspecies hybrids/varieties.
<https://www.vivc.de> (Vitis International Variety Catalogue, 2022)

Hybrid/ Variety	Variety number VIVC	Country or region of the variety origin	Pedigree as given by breeder	Year of breeding
Bronner	17129	Germany	Merzling x Geisenheim 6494	1975
Muscaris	22628	Germany	Solaris x Gelber Muskateller	1987
Morava	23777	Serbia	[(Kunbarat x Tramin) x Bianca)) x Rajnai Rizling GM 239-20	-
Pinot Blanc	9272	France	Pinot Noir Mutation	-

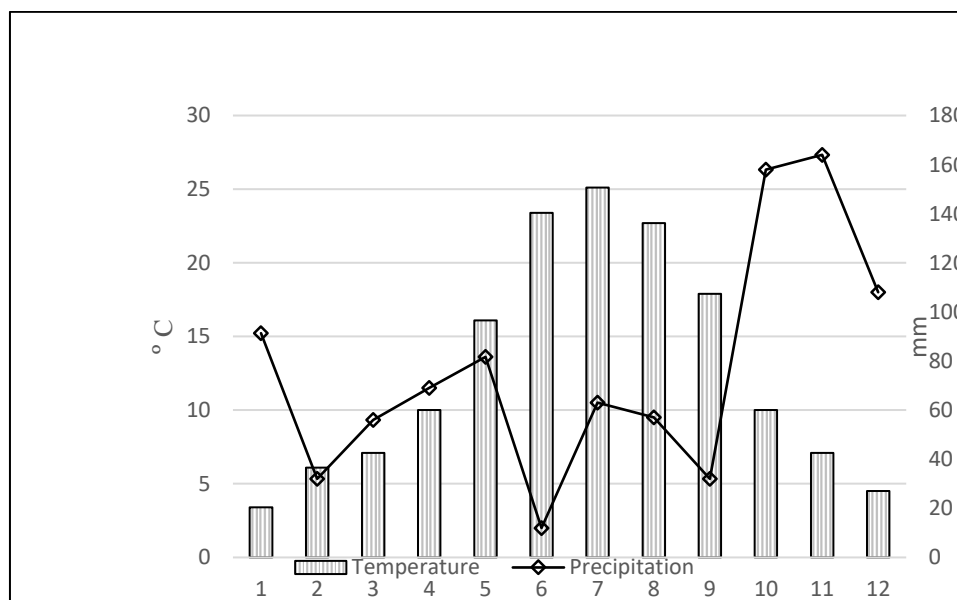


Figure 1. The average of monthly air temperature (°C) and monthly total precipitation (mm) (Banja Luka, 2021)

In general, the area of northern Bosnia, where the Banja Luka region is located (the valley of the river Vrbas), has a continental and moderately continental climate (Republic of Srpska Hydrometeorological Institute, 2022). Meteorological conditions in the year of the research were of a rather moderate character, excluding the occurrence of a significantly higher amount of precipitation in September (Figure 1).

Ampelographic characteristics (grape cluster weight, weight of 100 grape berries, skin and seed weight of 100 grape berries and number of seeds in 100 grape berries) were analyzed on a sample of 10 representative grape clusters, i.e. 100 grape berries, according to the Prosteserdov methodology, 1946. The basic technological characteristics of the tested varieties were evaluated on based on the grape juice analysis: the percentage of total soluble solids - sugar ($^{\circ}$ Brix), total titratable acidity and pH value of grape juice. The content of total soluble solids - sugar ($^{\circ}$ Brix) was measured with a digital refractometer (Atago Pal-3), the must total titratable acidity (TTA) was determined by potentiometric titration with neutralization method using 0.1 M NaOH solution (g tartaric acid/L) and the pH value was determined by using a pH meter (HannaHI 2211). In addition, an analysis of the phenolic compounds of the tested samples of the varieties was also performed. The determination of total polyphenols content in the skin and seeds of grapes was carried out according to the protocols applied in the research within the framework of the COST Action FA1003 project, "East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding" (Rustioni et al. 2014, Di Stefano et al. 1989), by measuring the absorbance of the extract at 700 nm with a spectrophotometer UV/VIS mini-1240, Shimadzu. The total polyphenols content in the extract of grape skins and seeds was expressed as (+) catechin concentration ($\text{mg}\cdot\text{L}^{-1}$), and then converted to $\text{mg}\cdot\text{kg}^{-1}$ of grapes. All data were analyzed in Microsoft Office Excel by applying two-factorial analysis of variance (ANOVA). All values in tables for parameters are shown as arithmetic mean with standard error ($\bar{X} \pm \text{Se}$) and coefficient of variation. Significant interaction effects were identified and further analyzed. Duncan's test was used to evaluate the pomological and chemical data and to detect significant differences ($p \leq 0.05$) between the mean values.

RESULTS AND DISCUSSION

Ampelographic characteristics

The ampelographic characteristics of grape cluster and grape berries of analyzed varieties are presented as average values of 10 grape clusters, i.e. 100 grape berries, in Tables 2, 3 and 4.

Table 2. Grape cluster weight (g) and number of grape berries per grape cluster of analyzed varieties

	Grape cluster weight (g)			Number of grape berries per grape cluster		
	\bar{x}	S \bar{x}	Vc	\bar{x}	S \bar{x}	Vc
Bronner	192.62 ^{bc}	13.36	21.94	157.80 ^a	9.79	19.61
Morava	169.44 ^c	9.26	17.29	106.70 ^b	5.57	16.51
Muscaris	244.99 ^a	9.50	12.27	146.30 ^a	5.95	12.86
Pinot blanc	222.63 ^{ab}	14.09	20.01	173.90 ^a	10.69	19.44

\bar{x} - mean values; S \bar{x} - standard error of mean; Vc – coefficient of variation

a-c: Different letters within the same column indicate statistically significant difference at $p < 0.05$ by Duncan's test

The obtained results for the grape cluster weight of analyzed varieties (Table 2) are ranged from 169.44 g (Morava variety) to 244.99 g (Muscaris variety). The result for Muscaris variety grape cluster weight was statistically higher compared to the results for other observed varieties, with the exception of the Pinot Blanc variety, that was not statistically different from the Bronner variety in terms of grape cluster weight. The variety Morava had the lightest grape cluster among the observed varieties. Grape cluster weight was fairly uniform, as indicated by the coefficient of variation for this characteristic.

The difference in the number of grape berries (Table 2), excluding variety Morava, did not differ statistically significantly in the mutual comparison of the other analyzed varieties. The highest number of grape berries per grape cluster was recorded in the variety Muscaris (173.90) and the lowest in the variety Morava (106.70).

The variety Muscaris had a statistically significantly higher weight of 100 grape berries (173.06 g) compared to the other analyzed varieties (Table 3). The variety Morava had a slightly higher weight of 100 grape berries (151.47 g) compared to the varieties Bronner and Pinot Blanc, among which no statistically significant difference in the weight of 100 grape berries was observed. A similar situation was observed with the grape skin weight of 100 grape berries. The grape skin weight of 100 grape berries ranged from 9.79 g (Bronner) to 17.54 g (Muscaris). Compared to the results of a two-year study (2017-2018, Spain), Casanova-Gascón et al, 2019a, the weight of 100 Muscaris berries was lower (132.95 g and 128.30 g, respectively).

Table 3. Weight of 100 grape berries (g) and weight of the skin of 100 grape berries (g) of the analyzed varieties

	Weight of 100 grape berries (g)			Weight of the skin of 100 grape berries (g)		
	\bar{x}	S \bar{x}	Vc	\bar{x}	S \bar{x}	Vc
Bronner	131.07 ^c	3.71	8.94	9.79 ^c	0.33	10.62
Morava	151.47 ^b	5.68	11.86	12.39 ^b	0.45	11.47
Muscaris	173.06 ^a	4.95	9.05	17.54 ^a	0.63	11.30
Pinot blanc	138.24 ^{bc}	3.22	7.37	10.55 ^{bc}	0.44	13.15

\bar{x} - mean values; S \bar{x} - standard error of mean; Vc – coefficient of variation

a-c: Different letters within the same column indicate statistically significant difference at $p < 0.05$ by Duncan's test

The weight of seeds in 100 grape berries (Table 4) was quite uniform in all varieties, except for the Pinot Blanc variety (7.63g), which had a statistically significantly lower weight compared to the other tested varieties.

Table 4. Weight of seeds in 100 grape berries (g) and number of seeds in grape 100 berries

	Weight of seeds in 100 grape berries (g)			Number of seeds in 100 grape berries		
	\bar{x}	S \bar{x}	Vc	\bar{x}	S \bar{x}	Vc
Bronner	10.35 ^a	0.41	12.40	321 ^a	9.53	9.39
Morava	10.78 ^a	0.74	21.61	219 ^b	11.87	17.14
Muscaris	11.50 ^a	0.37	10.24	300 ^a	8.37	8.82
Pinot blanc	7.63 ^b	0.33	13.49	216 ^b	5.69	8.83

\bar{x} - mean values; S \bar{x} - standard error of mean; Vc – coefficient of variation

a-b: Different letters within the same column indicate statistically significant difference at $p < 0.05$ by Duncan's test

In the other varieties, an uniform weight of seeds in 100 grape berries was observed, ranging from 10.35 g (Bronner) to 11.50 g (Muscaris). The number of seeds in 100 grape berries was statistically significantly lower in the varieties Pinot Blanc (216) and Morava (219) compared to the varieties Muscaris (300) and Bronner (321), which did not differ from each other.

Technological characteristics

The results of the analysis of technological characteristics of the studied varieties grapes are presented in Figure 2 and Table 5.

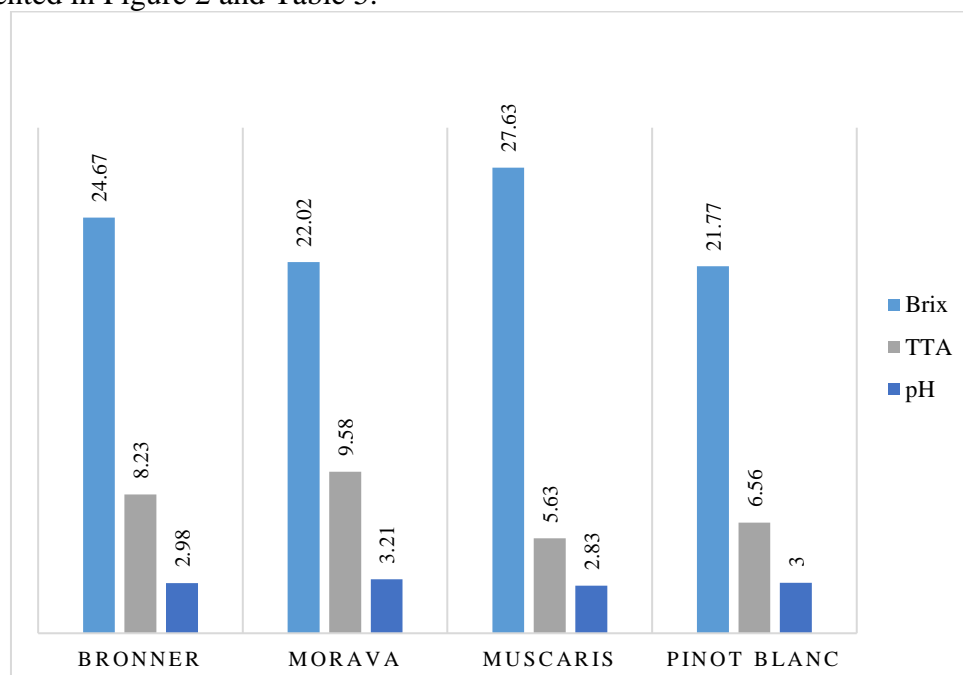


Figure 2. °Brix, TTA (g/L) and pH value of the analyzed varieties

Based on the presented results of the analysis of the technological characteristics of the studied varieties with histogram (Figure 2), it is visible that the must of Muscaris variety had the highest sugar content (27.63 °Brix), while the lowest content was observed in the Pinot Blanc variety (21.77 % °Brix). According to the OIV codes, the interspecific hybrids Bronner and Muscaris have a very high sugar content, the Pinot Blanc variety has a high sugar content, while the Morava has a sugar content between high and very high. Regarding the must total titratable acidity, interspecific hybrid Morava's must had the highest total acidity (9.58 g/L), while the lowest was observed in the must of interspecific hybrid Muscaris (6.3 g/L). According to the OIV codes, the must of Muscaris and Pinot Blanc varieties had a low total acidity, Bronner had high, while Morava was classified in the category between high and very high total acidity. According to the results of research carried out in Poland, in colder climate conditions (Czaplicka et al., 2022), the sugar content in Muscaris hybrid was 22.0 °Brix. The average results of a three-year study (2015-2017) conducted in northern Italy (Pedò et al., 2019) showed that the sugar content in the must of interspecific hybrid Muscaris was 23.7 °Brix, and total titratable acidity 6.53 g/L, while in interspecific hybrid Bronner's must, the sugar level was observed to be 20.4 °Brix, and total titratable acidity 6.33 g/L. Porro et al., 2019 reported similar results of the sugar content in must of Muscaris and Bronner (Muscaris 24.17 °Brix, Bronner 20.19 °Brix), grown in the northeastern region of Italy (2013). In contrast to the above mentioned, according to the results of research conducted in Spain (Casanova-Gascón et al., 2019b), the acid level in the must of Muscaris variety was significantly lower, 3.79 g/L in 2017, and 3.79 g/L in 2018. A four-year study (1995-1999) conducted in northern Bavaria, Germany (Schwab et al., 2000) and a two-year study (2004-2005) in Switzerland (Tuchschnid et al., 2005) found that the must of interspecific hybrid Bronner had an approximate content of sugar as in the aforementioned growing conditions of this variety in northern Italy, 20.6 °Brix, i.e. 19.6-22.5, °Brix, while the total titratable acidity was 10.1 g/L, i.e. 7.3-9.6 g/L. According to

published data, the interspecific hybrid Bronner, grown in the warm climate of Brazil, had a slightly lower sugar content in the must, 19.0 Brix (de Bem et al., 2016a).

According to Guguchkina et al., 2020, the sugar content in the must of interspecific hybrid Morava, in the Krasnodar region of Russia, ranged from 18.3-21.5 °Brix, and total acidity from 8.9 to 11.5 g/L., while the sugar content in the same hybrid grown conventionally (2015-2018) in Sremski Karlovci, was 20.54 °Brix, and total titratable acids were 7.34 g/L (Ivanišević et al., 2022). The Pinot Blanc variety, grown in Sremski Karlovci (Serbia), according to multi-year research data, 1981-1988 (Cindrić, et al., 2000), had an average sugar content of 18.5 °Brix, and total titratable acidity of 8.8 g/L.

Based on all the above, it can be said that the sugar content in our analyzed interspecific hybrids, Muscaris and Bronner, was higher compared to the results of other authors, while the sugar level of Morava was relatively in line with the published statements. The comparative results of the must total titratable acidity show that the acidity of the Bronner’s must was relatively close to the must acidity of the mentioned interspecific hybrid grown in the conditions of northern Germany and Switzerland, while the lower acidity values of the must of interspecific hybrid Muscaris were relatively in accordance with the measured acidity values of the said hybrid, grown in northern Italy.

The obtained must pH values of the interspecific hybrids Muscaris and Bronner in our research are in line with the research of other authors. According to the data of Czaplicka et al., 2022b, the pH value of the Muscaris must was 2.92, while in our research it was 2.83. According to our analysis results, the must pH value of the interspecific hybrid Bronner was 2.98, which is in line with de Bem et al., 2016b, where the pH value was 2.96. The exception to the above are the results of the research conducted by Casanova-Gascón et al, 2019c, where the must pH value of the interspecific hybrid Muscaris was higher compared to our research, i.e. 3.15 in 2017 and 3.71 in 2018.

Table 5. Content of total polyphenols in the skin and content of total polyphenols in the seed (mg/kg)

	Content of total polyphenols in the skin (mg/kg)			Content of total polyphenols in the seed (mg/kg)		
	\bar{x}	S \bar{x}	Vc	\bar{x}	S \bar{x}	Vc
Bronner	1876.73 ^a	14.65	2.34	1881.26 ^b	38.42	6.13
Morava	742.98 ^c	11.57	4.67	847.30 ^d	16.54	5.85
Muscaris	1072.76 ^b	32.90	9.20	1582.06 ^c	55.11	10.45
Pinot blanc	1060.58 ^b	6.11	1.73	2913.68 ^a	27.15	2.80

\bar{x} - mean values; S \bar{x} - standard error of mean; Vc – coefficient of variation

a-b: Different letters within the same column indicate statistically significant difference at $p < 0.05$ by Duncan’s test

The content of total polyphenols in the skin was statistically significantly higher in the Bronner variety (1876.73 mg/kg) compared to the other tested varieties. A fairly uniform content of total polyphenols in the skin was observed in the interspecific hybrid Muscaris (1072.76 mg/kg) and the variety Pinot Blanc (1060.58 mg/kg). The lowest content of total polyphenols was found in the skin of the variety Morava (742.98 mg/kg). The content of total polyphenols in the seeds was statistically significantly different in the analyzed varieties in a mutual comparison. The highest values were obtained in the Pinot Blanc variety (2913.68 mg/kg), and the lowest in the Morava variety (847.30 mg/kg). The obtained results are in line with the results of the research conducted in Turkey (Atak et al., 2021), where the content of phenolic compounds (catechin and epicatechin) in the analyzed 5 interspecific hybrids and 17 varieties of *V. vinifera* was higher in the grape seeds compared to the skin and pulp. Although

the average weight of 100 grape berries, skins and seeds was the highest in the Muscaris variety, the content of total polyphenols in the skin and seeds was not, which is in contrast to the results of the research by Pascale-Gagne et al., 2016, where the size of the grape berry (weight and diameter) and a higher relative proportion of the skin and seeds in the structure of the grape berry of the studied disease-resistant varieties, positively influenced the content of total phenols in the skin and seeds.

CONCLUSIONS

Based on the evaluation of the ampelographic and technological characteristics of the analyzed interspecific hybrids of Bronner, Muscaris and Morava and comparative analysis with the commercial white wine variety Pinot Blanc, it was concluded that the grapes of the hybrid Muscaris had the best ampelographic characteristics, followed by the grapes of Bronner and Morava. Based on the must analysis, it can be said that the interspecific hybrids Muscaris and Bronner stood out with a high sugar content, higher even compared to the results of other mentioned authors, and that the must quality of the Morava variety was at a satisfactory level. Since there is considerably less data in the literature regarding the phenolic compounds content in interspecific hybrid grapes, compared to commercial varieties, only a general assessment of the phenolic status was possible, according to which the content of total polyphenols was higher in the seeds compared to the skin.

The fact that the research so far has shown satisfactory potential and quality of all analyzed interspecific hybrids indicates the need to continue research in order to more comprehensively determine their agro-technological potential.

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