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Morphological, pomological, and nutritional value of wild and cultivated rosehip (*Rosa canina* L.) genotypes in Slavonia, Croatia

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Abstract: In this study, the morphological, pomological, and nutritional values of wild and cultivated rosehip fruits grown in the Slavonia region of eastern Croatia were studied. The results revealed significant differences in several morphological and pomological characteristics among the rosehip genotypes in terms of fruit weight, flesh weight, seed weight, and fruit flesh ratio, with no significant differences in fruit width, fruit length, fruit shape index, seed number per fruit, or seed length. The evaluated rosehip fruit genotypes differed significantly from each other in terms of hectoliter weight (kg), fruit bulk (cm³), and bulk density (kg/m³). For water-soluble extracts, ash, and pH, no statistical difference was found between naturally grown genotypes, but there was a significant difference between naturally grown and cultivated genotypes. Twenty-three major and trace elements were analyzed. The most abundant elements were K, Ca, Mg, and P in both cultivated and naturally grown fruits. The highest concentrations of microelements were Fe, Al, Mn, and Sr. The conventionally cultivated genotype L1 had the highest concentration of Fe and Na as essential elements for humans but also had the highest concentrations of Al, Sr, Ti, V, Cr, Pb, Co, Li, and As of all the genotypes studied. The naturally grown genotype L4 had the highest

concentrations of S, Zn, Rb, and Cd and the lowest concentrations of Mg, K, and Ca among all studied genotypes. The data showed that the analyzed genotypes from eastern Croatia had good nutritional quality and variability, making them suitable as genetic resources and possibly leading to the detection of rosehip genotypes as potential sources of beneficial ingredients for human health.

Keywords: rosehip; wilderness grown; cultivated; nutritional value

Running head: Morphological, pomological, and nutritional value of rosehip genotypes

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1. Introduction

The genus *Rosa* includes approximately 200 species (Żuraw et al., 2015) and many *Rosa spp.* grow along roadsides, the edge of woods, and other wild places in the northern hemisphere only. In Croatia, the most widespread wild species is rosehip (*Rosa canina* L.); however, the cultivation of rosehip fruit in Croatia is almost nonexistent (Šindrak et al., 2012). The consumption of rosehip fruit is very popular in Scandinavian countries, Germany, and Eastern European countries (Patel, 2017). To the best of our knowledge, no scientific studies on the morphology and nutritional value of rosehip species grown in eastern Croatia have been published. Growing rosehips is important because of their potential value in organic farming, biodiversity conservation, environmental protection, and the nutritional and medicinal properties of their fruits. The fruits of the dog rose to have high phenolic (Hvattum, 2002), vitamin C (Demir & Ozcan, 2001; Chrubasik et al., 2008), and carotenoid (Hornero-Mendez & Minguéz-Mosquera, 2000) content, and also contain folates, calcium, potassium, phosphorus, and other vitamins and minerals (Szentmihályi et al., 2002; Hakki Yoruk et al., 2008). Because of their natural antioxidant activity and beneficial effects on the human body, they are used in health protection (Demir et al., 2014; Smanalieva et al., 2020) and for food production, such as tea, jams, and marmalades (Yildiz & Alpaslan, 2012). In general, fruit species found in spontaneous flora have always been used for both food and medicinal purposes because of their high bioactive compound content (Mármol et al., 2017; Cosmulescu et al., 2020).

The aim of this study was to evaluate and characterize the morphological, pomological, and nutritional value of the chemical and mineral content in rosehip fruits and pulp from the genotypes of cultivated and naturally grown *Rosa canina* L. plants in Slavonia, eastern Croatia.

2. Material and methods

This study was conducted in eastern Croatia in September 2020, and samples were taken from four different locations (L1 – Oriovac, 49°9'59.96" N, 17°44'41.82" E; L2 – Slobodnica, 45°09'58.5" N, 17°56'52.9" E; L3 – Sapna, 45°21'40.17" N, 18°2'7.13" E; L4 – Grgurevići, 45°13'56.88" N, 17°53'11.33" E). *Rosa canina* var. *inermis* was grown at location L1 with conventional fruit growing methods; *Rosa canina* var. 'Brogs Stachellose' were grown with organic methods at location L2, and a fruit selection was naturally grown at locations L3 and L4. All four locations have a moderate continental climate, with an average monthly temperature above 10 °C for more than four months, a medium temperature below 22 °C in the hottest month, and an average annual rainfall of 700 – 800 mm. The areas along the Sava River and its surroundings have predominantly alluvial-amphigley soils, with occasional excessive wetting by surface water (pseudogley). The rosehip samples consisted of one hundred mature fruits at the same ripening stage (intense red color) from ten plants in four repetitions that were randomly selected. At each location, the samples were randomly harvested from different shrub heights at the optimal maturity stage. The samples were transferred to the Agroecological Laboratory, Biotechnical Department at the University of Slavonski Brod in Croatia and stored in a cooler until the morphological, technological, chemical, and mineral (lyophilized and ground hips) analyses at the Technology Laboratory, Polytechnic in Požega, Požega, Croatia and Laboratory of Ruder Boskovic Institute in Zagreb, Croatia. All analyses were performed within three weeks. The samples were assessed for characteristics such as fruit length (mm), width (mm), fruit weight (g), fruit shape index (FSI - ratio between the fruit height (length) and the fruit diameter), flesh weight (g), stone number/fruit, stone length (mm), stone weight (g), fruit flesh ratio (%), hectoliter weight (kg), fruit bulk (cm³), bulk density (kg/m³), dry matter content (%), water-soluble extract (%), ash (%), pH acidity (%), malic acid, total polyphenols (mg GAE/100 g), and antioxidant activity

(AA). The total concentrations of the following elements were determined: P, Na, Mg, K, Ca, S, Mn, Fe, Cu, Zn, Co, Cr, Rb, Pb, Al, Ba, Ni, Sr, As, Li, Cd, Ti, and V. The fruit was weighed using a Nimbus analytical balance NBL 254 I scale (Adam Equipment, Kingston, UK), and the fruit length and width were measured using a DIGI-MET 1226932-D sliding scale (Helios Preisser, Gammertingen, Germany).

Extract preparation

Pulp (1 g) was extracted using 20 mL of acidified methanol (methanol/2% HCl, 95:5) at 20 °C for 60 min with consistent shaking in a temperature-controlled shaker (Kottermann Labortechnik Köttermann GmbH, Uetze, Germany) at 200 rpm and centrifuged (Centric 322A, Tehnica, Domel d.o.o., Železniki, Slovenia). The glasses were covered with aluminum foil to prevent the solvent from evaporating.

Total phenol content

Polyphenol content was determined using the Folin–Ciocalteu method (Obradović et al. 2015). An aliquot of the extract (200 µL) was mixed with 2 mL water and 100 µL Folin–Ciocalteu reagent (Kemika, Zagreb, Croatia). The mixture was allowed to equilibrate for 5 min, after which 300 µL of sodium carbonate solution (20%) was added. After incubating at room temperature for 30 min in the dark, the absorbance of the mixture was recorded at 725 nm (UV-VIS Spectrophotometer, M501, Camspec, Ballyclare, UK). Acidified methanol was used as a blank. The total polyphenol content was determined using three replicates. Gallic acid (Carlo Erba reagents, Milano, Italy) was used as a standard (calibration curve $y = 1.1979x - 0.0188$, $R^2 = 0.9984$), and the results were expressed in mg of gallic acid equivalents per 100 g of sample.

Antioxidant activity (AA) determination by stable free radical diphenyl picrylhydrazyl (DPPH method)

An aliquot of the extract (50 µL) was mixed with 2 mL DPPH radical solution (0.1 mM in ethanol). The absorbance of the mixture was recorded at 517 nm over a period of 30 min, and the results were expressed as the mean of three replicates. Pure ethanol was used as a blank.

AA as % inhibition was calculated according to the following equation:

$$\% \text{ inhibition} = \frac{A_0 - A_t}{A_0} \times 100$$

where A_0 is the absorbance of the DPPH radical solution and A_t is the absorbance after 30 min.

Total acids

The determination of acidity (total acids) was performed by titration with 0.1 M NaOH solution, using phenolphthalein as an indicator and expressed as malic acid.

Soluble dry matter

Refractometer model Abbemat 3100, Anton Paar, Graz, Austria, method ISO 2173: 2003.

pH value

(pH meter model: Model pH 213, HANNA Instruments, Woonsocket, Rhode Island, USA; method ISO 1842:1991).

Ash

(ISO 5984 method).

Dry matter

The proportion of total dry matter by drying to constant weight at 105 °C.

Multi-element analysis using plasma mass spectrometry

Pulp samples from each location were subjected to multi-element analyses. Before analysis, the samples were lyophilized, ground in an agate mortar, and dissolved in a closed microwave system according to the method described below. The sample resolution was performed using a Multiwave ECO microwave system (Anton Paar, Graz, Austria). Initially, 0.05 g of sample was weighed, after which 7 mL of HNO₃ (65% supra pur, Fluka, Steinheim, Switzerland) and

0.1 mL of HF (48%, pro analysis, Kemika, Zagreb, Croatia) were added to the samples. After degradation, the samples were acidified with 2% (v/v) HNO₃ (65% supra pur, Fluka, Steinheim, Switzerland) without further dilution, and indium (In, 1 µg L⁻¹) was added as an internal standard.

High-resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) was used to determine the total concentrations of P, Na, Mg, K, Ca, S, Mn, Fe, Cu, Zn, Co, Cr, Rb, Pb, Al, Ba, Ni, Sr, As, Li, Cd, Ti, and V.

The Element 2 HR-ICP-MS instrument (Thermo, Bremen, Germany) was used, and details of the instrumental parameters are provided in Fiket et al. (2017).

Statistical analysis

Data were analyzed according to the random distribution scheme by one-way analysis of variance using the Least Significant Difference test (LSD test) with a significance level of $p < 0.05$ in the Statistica 12.0 statistical program.

3. Results

The evaluated rosehip fruit genotypes from four different locations and cultivation methods were not significantly different in fruit length, fruit width, fruit shape index, seed number per fruit, and seed length but did differ significantly in fruit weight, flesh weight, seed weight, and fruit flesh ratio (Table 1). Genotype L1 had a significantly higher flesh weight than the other genotypes. Genotypes L1 and L2 had significantly higher fruit flesh ratios than L3 and L4. Genotypes L1 and L4 had significantly higher fruit weights than genotypes L2 and L3. Genotypes L3 and L4 had significantly higher seed weights than L2, whereas the L1 genotype had no significant differences in seed weight for all three genotypes (Table 1).

Some of the pomological and phytochemical characteristics of the rosehip genotypes are listed in Table 2. The evaluated fruit genotypes of rosehip were significantly different ($p <$

0.05) from each other in bulk density and total polyphenol. Bulk density was significantly higher in the L3 genotype and significantly lower in the L1 genotype. The total polyphenol was significantly higher in the L4 genotype and significantly lower in the L1 genotype. The total polyphenol was significantly higher in the L4 genotype and significantly lower in the L1 genotype. An evaluation of the dry matter content, acidity (% malic acid), and antioxidant activity showed statistically significant differences between the L1 genotype and the other three genotypes; however, no statistical differences were between the L2, L3, and L4 genotypes. The L1 genotype had the highest acidity but the lowest dry matter content and antioxidant activity (Table 2).

For the water-soluble extract, ash, and pH, no statistical difference was found between naturally grown (L3 and L4) genotypes but was found between naturally grown (L3, L4) and cultivated genotypes (L1, L2), and naturally grown fruits had the highest value for the mentioned attributes (Table 2). The total polyphenol compounds of rosehip genotypes changed significantly depending on the genetic variation (Table 2). The highest and lowest levels of total polyphenol compounds were detected in L4 (4634.43 mg GAE/100 g DW) and L1 (4033.37 mg GAE/100 g DW) samples, respectively.

Twenty-three major and trace elements were analyzed (Table 3) and the results showed significant differences between the rosehip genotypes at different locations. The most abundant elements detected in this study were K and Ca; however, Ca was less abundant in wild rosehip fruit than in the cultivated rosehip genotypes. The highest concentrations of microelements were Fe, Al, Mn, and Sr, whereas the lowest concentrations of microelements were As, Co, Cd, Pb, and Cr (Table 3). Genotypes L4 and L1 had higher Fe and Na content, which are essential elements for humans, and higher contents of Al and Ti. The L1 genotype also had higher contents of Sr, V, Cr, Pb, Co, Li, and As. The other genotypes that were examined had similar results for the aforementioned elements. This may be because

conventional fruit grows with different chemical methods of plant protection, types of fertilization, and locations. Genotype L4 had higher concentrations of S, Zn, Rb, and Cd and the lowest concentrations of Mg, K, and Ca, which are essential elements for humans. The naturally grown L3 genotype had smaller fluctuations in mineral content than the other two cultivated genotypes.

4. Discussion

The results of this rosehip fruit genotype analysis corresponded with other studies that recorded similar pomological properties, such as fruit length, fruit width, fruit weight, fruit shape index, flesh weight, number of seeds per fruit, seed length, seed weight, and fruit flesh ratio (Dogan & Kazankaya, 2006; Stoenescu & Cosmulescu, 2021). Statistical differences between cultivated species and genotypes in naturally grown locations were observed for seed weight and fruit flesh ratio, indicating that the genotypes from naturally grown locations had a smaller proportion of fruit flesh and higher seed weight. For most of the studied morphological and pomological traits, the rosehip fruit genotype L3 from the naturally grown area showed the lowest values (Table 1). Demir & Ozcan (2001) reported average fruit length values of 17.29 mm to 19.68 mm for rosehip in Turkey. Rosu et al. (2011) reported fruit lengths between 11.40 mm and 30.90 mm in Romania.

The rosehip fruit genotypes differed significantly from each other in fruit weight, flesh weight, seed weight, and fruit-flesh ratio (Table 1). Similar results were reported in other studies when comparing the morphological and pomological characteristics of rosehip fruits (Erogul & Oguz, 2018; Fascella et al., 2019). Ercisli & Guleryuz (2006) determined that the promising selection of rosehip exhibited a fruit-flesh ratio range of 61.67–74.20 %. In this study, all four genotypes were promising selections recorded by Ercisli & Guleryuz (2006); however, statistical differences were found between naturally grown (L3 and L4) and

cultivated (L1 and L2) rosehip fruits. For hectoliter weight and bulk density, genotype L1 had the smallest value (58.15 kg; 581.53 kg/m³) and genotype L3 had the highest value (61.95 kg; 619.47 kg/m³) (Table 2). High dry matter content, water-soluble extracts, and acidity levels are desirable characteristics for rosehip fruits used in the processing industry to obtain better quality marmalade, jam, jelly, or herbal tea (Dogan & Kazankaya, 2006; Ercisli, 2007). These identified fruit characteristics similar to those identified previously (Kazankaya et al., 2005; Demir et al., 2014).

Previous studies have reported that the phytochemical characteristics of rosehip fruit could be influenced by various factors, such as genotype, cultivar, environmental conditions, growth conditions, region, harvest time, and maturation stage (Çelik et al., 2009; Ipek & Balta, 2020). Statistical differences were found between rosehip fruit genotypes for total polyphenol compounds; however, the genotypes from naturally grown fruits (L3, L4) had higher values than the genotypes from cultivated rosehip fruit (L1, L2). The rosehip genotype, region, differences in fruit ripeness, and extraction technique can affect the total polyphenol compounds in the fruit, which is similar to the findings of previous studies (Su et al., 2007; Demir et al., 2014; Fescella et al., 2019).

Twenty-three macro- and microelements were analyzed (Table 3), and the results showed significant differences between the rosehip genotypes at the different locations. Demir and Ozcan (2001) reported similar results. The most abundant elements detected in this study were K, Ca, Mg, and P for cultivated and naturally grown fruits, which is comparable with the findings of previous studies (Popović-Djordjević et al., 2021; Ercisli, 2007). Popović-Djordjević et al. (2021) determined that Ca, Cu, K, Mg, Mn, and P found in rosehip fruit are good sources of essential elements needed for human nutrition. The concentrations of P, Ca, and S were uniform in the cultivated rosehip fruits; this was in contrast to the wild growth, in which the concentrations fluctuated considerably. The conventionally grown L1 genotype had

the highest concentrations of Na, Co, Sr, As, Li, V, Pd, and Cr, which could be caused by the use of chemical protective agents, mineral fertilization, or other sources of anthropogenic origin. Kalinović et al. (2019) concluded that *Rosa* spp. has the potential for use in biomonitoring.

5. Conclusion

An analysis of morphological, pomological, and nutritional values revealed variability in rosehip genotypes as a result of ecological, cultivation, and hereditary factors. The genotype variability of rose hip fruit grown naturally, conventionally, and organically in Slavonia indicates the potential for this plant to be further studied in this part of Croatia to establish a connection between fruit material and the influence of various factors, such as location, variety, growing method, and ecological conditions. Further research on the pomological and chemical composition of rosehip fruits is needed so that they can serve as effective morphological, pomological, and genetic resources, especially the L3 genotype.

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Author's contributions:

TBL, KM – design of the experiments, writing of the manuscript

BJP, MP, RB – field experiments and analytical measurements in the laboratory

VO – analytical measurements in the laboratory

MR, RB – statistical analysis of experimental data

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Table 1. Morphological and pomological characteristic of genotypes of rosehip fruits

| Genotypes | Fruit weight (g) | Fruit length (mm) | Fruit width (mm) | Fruit shape index | Flesh weight (g) | Seed number per fruit | Seed length (mm) | Seed weight (g) | Fruit flesh ratio (%) |
|-----------|------------------|-------------------|------------------|-------------------|------------------|-----------------------|------------------|-----------------|-----------------------|
| L1 | 1.90a | 22.70a | 13.18a | 1.72a | 1.41a | 21.13a | 5.32a | 0.49ab | 74.25a |
| L2 | 1.70b | 21.95a | 13.15a | 1.67a | 1.25b | 21.93a | 5.14a | 0.42b | 74.99a |
| L3 | 1.67b | 21.49a | 13.07a | 1.64a | 1.12b | 22.42a | 5.18a | 0.59a | 64.71b |
| L4 | 1.82a | 22.18a | 13.28a | 1.67a | 1.25b | 23.77a | 5.39a | 0.58a | 68.32b |

Different letters within the same column indicate statistically significant differences between different rosehip genotypes ($p < 0.05$). L1–L4 are genotypes from four different locations.

Table 2. Pomological and phytochemical characteristic of genotypes of rosehip fruits

| Genotypes | Hectoliter weight (kg) | Fruit bulk (cm ³) | Bulk density (kg/m ³) | Dry matter content (%) | Water soluble extract (%) | Ash (%) | pH | Acidity (% malic acid) | Total polyphenols (mg GAE/100g DW) | AA |
|-----------|------------------------|-------------------------------|-----------------------------------|------------------------|---------------------------|---------|-------|------------------------|------------------------------------|--------|
| L1 | 58.15c | 1.0535a | 581.53d | 36.60b | 28.84b | 1.84b | 3.49b | 0.44a | 4033.37d | 69.11b |
| L2 | 60.88b | 1.0409b | 608.77b | 41.34a | 28.77b | 0.93c | 3.41b | 0.31b | 4238.75c | 86.79a |
| L3 | 61.95a | 1.0191c | 619.47a | 43.44a | 33.11a | 2.29a | 3.74a | 0.31b | 4447.35b | 83.38a |
| L4 | 60.04b | 1.0404b | 600.37c | 42.62a | 34.03a | 2.37a | 3.72a | 0.29b | 4634.43a | 81.59a |

Different letters within the same column indicate statistically significant differences between different rosehip genotypes ($p < 0.05$). L1–L4 are genotypes from four different locations.

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Table 3. Concentrations of macro and micro elements in rosehip fruits genotypes

| Element concentration (ppm) | Genotypes | | | |
|-----------------------------|-----------|-------|-------|-------|
| | L1 | L2 | L3 | L4 |
| P | 1329 | 1364 | 1848 | 1284 |
| Na | 91.1 | 22.9 | 31.5 | 26.2 |
| Mg | 1965 | 2587 | 2185 | 1410 |
| K | 18367 | 15575 | 18383 | 9386 |
| Ca | 8934 | 8458 | 7107 | 2856 |
| S | 460 | 461 | 470 | 662 |
| Mn | 14.8 | 37.5 | 24.1 | 5.29 |
| Fe | 31.8 | 26.1 | 25.6 | 33.2 |
| Cu | 4.56 | 2.56 | 2.79 | 4.37 |
| Zn | 11.1 | 8.09 | 6.59 | 29.6 |
| Co | 0.17 | 0.02 | 0.04 | 0.02 |
| Cr | 0.91 | 0.13 | 0.14 | 0.13 |
| Rb | 11.73 | 16.94 | 2.62 | 19.46 |
| Pb | 0.56 | 0.13 | 0.24 | 0.21 |
| Al | 44.2 | 36.7 | 35.3 | 45.7 |
| Ba | 9.07 | 10.53 | 13.63 | 3.09 |
| Ni | 1.7 | 1.08 | 1.24 | 1.81 |
| Sr | 31.56 | 20.27 | 19.2 | 11.14 |
| As | 0.1 | 0.01 | 0.02 | 0.02 |
| Li | 0.42 | 0.03 | 0.05 | 0.05 |

| | | | | |
|----|-------|-------|-------|-------|
| Cd | 0.016 | 0.005 | 0.003 | 0.048 |
| Ti | 3.72 | 2.35 | 2.6 | 3.51 |
| V | 0.93 | 0.06 | 0.05 | 0.08 |

Calculated as mg per kg fresh fruit. L1–L4 are genotypes from four different locations.

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