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Article

Selection of Mulberry Genotypes from Northern Serbia for 'Ornafruit' Purposes

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Abstract: The genus *Morus* L., mulberry, is an interesting taxonomic group on account of its existing genetic variability, functional food potential and commercial importance. Mulberry trees are found in a wide range of areas in Serbia, accounting for a large phenotypic diversity in its genetic resources. Tree and fruit characteristics of more than 300 mulberry specimens were surveyed, and 15 genotypes of *Morus alba*, *Morus nigra* and *Morus rubra* species were selected for further analyses. These were located at various sites in the province of Vojvodina, Serbia. The present study was undertaken to investigate the diversity of the collected material aiming to pre-select genotypes suitable for landscaping/ornamental and/or fruit production purposes. Genotypes BP 3/9, DT1, ZP3 and MR1 have semi-vigorous growth, dropping growth habits, different leaf shapes (ovate, oval, cordate) and leaf color (from light to dark green), corresponding to ornamental mulberries. In addition, the semi-vigorous genotype ZD1 with a spreading tree and interesting palmate-lobed leaves was distinguished as a unique genotype for landscaping purposes. The most vigorous annual shoot growth was detected in the ZP3 genotype (118.5 cm), followed by DT1 (108.2 cm), MR1 (101.8 cm) and ZP1 (100.5 cm) genotypes. Contrary, genotype DJ1 exhibited the lowest annual growth with only a 32.9 cm average length of the shoots. Due to the greater fruit mass (4.2–6.1 g), sweetness and acidity balance as well as chemical composition, genotypes BP 1/4, DJ1, MG, MR1, DT1 and ZP3 may be recommended for fresh consumption, while genotypes DJ1, DT1, MR1, ZD1, ZP1 and BP 3/9 could be appropriate for home processing. According to fruit chemical analyses, the most promising genotypes were MR1 and DT1 combining high soluble solids content (21.2% and 18.5%, respectively), total sugar content (17.41% and 15.20%, respectively) and ascorbic acid content (42.24 and 49.28 mg/%, respectively). Additionally, DT1 genotype was also characterized by the highest total phenolic content (221.08 mg 27 GAE/100 g fresh weight). The most ornamental genotypes from this study (BP 3/9, DT1, ZD1, ZP3 and MR1) combined with their pomological and chemical characterization can be recommended for edible gardening purposes due to both aesthetic appearance and nutritive value of the fruits.

Keywords: mulberry; genetic diversity; morphometric traits; pomological traits; principle component analysis; chemical composition

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1. Introduction

A wide variety of underutilized fruit crops (including *Morus* sp.), which are neither commercially cultivated nor traded on a large scale, are mainly grown, commercialized and consumed locally [1]. Less known or underutilized fruit species are distributed in different parts of the world in temperate, subtropic and tropic conditions. They are attracting more and more popularity, and the number of studies on these species has increased, due to their edible as well as ornamental properties. These fruits often possess a high content of non-nutritive, nutritive, and bioactive compounds that are vital for human health. They are also accepted as healthy food because of the growth and harvest from natural populations or rural areas without the use of chemical fertilizers or pesticides. Exemplary studies include not only mulberries but carob tree (*Ceratonia siliqua* L.), honeysuckle (*Lonicera caerulea* L.) and wild sea buckthorn (*Hippophae rhamnoides* L.) that have distinct flavor and taste as well as excellent medical and aesthetic value [2–5].

Genus *Morus* is widespread in Asia, Europe, North and South America, and Africa. Mulberries can be found from between moderate and sub-tropical regions of the northern hemisphere, to the tropical regions of the southern hemisphere, because they can grow in a wide range of climatic, topographic and soil conditions. They are spread in all regions, from the tropics to the sub-Arctic, and from sea level to an altitude of 4000 m [1]. Mulberry is grown particularly in China, India, and Japan for sericulture, as the silkworms (*Bombyx mori* L.) feed exclusively on leaves of the *Morus* genus. In China, there are over a thousand mulberry cultivars available for sericulture originating from four main species, i.e., *M. alba*, *M. multicaulis*, *M. bombycis* and *M. atropurpurea.* Mulberry is also used as forage in animal production along with mainly fruit production for human consumption in Balkan countries [6,7]. In Turkey, mulberries have been cultivated for more than 400 years with predominant species of *Morus alba* (95%), 3% *M. rubra* (3%) and *M. nigra* (2%) [8], commonly eaten fresh, dried, or processed into molasses and jam for its delicious taste, pleasing color, low-calorie content, and high nutrient content [9].

The genetic diversity of mulberry in Northern Serbia, province of Vojvodina, is reflected in a large number of solitary trees that have never been investigated for their breeding potential. The expansion of mulberries in Vojvodina started in the XVII century. Later, former Yugoslavia was the fifth most important region for silk production with more than 2.5 million white mulberry trees during the 30 s of the XX century. In that time, in Serbia, mulberry trees were mainly grown for sericulture, but mulberry as an edible ornamental species was often planted in green belts, green corridors between villages, as well as in parks, alleys, or solitary trees in various green areas. The mulberry used for decorative purposes is often a grafted species with a hanging or pyramidal shape [10], interesting in landscape motives of urban and rural greenery. Coining the term 'ornafruits', Sahin [11] best described the potential of such greenery, referring to the fruit species that can be used for both fruit production and ornamental purposes. According to the same author, mulberries specifically can be used as a fast-growing tree cultivated for horticultural, pharmaceutical, industrial, and ornamental purposes, among which mulberries characterized by a weeping canopy are commonly used in landscape areas. In addition to the weeping form, *M. alba* var. *tortuosa* as a tortuous type can be applied as a small specimen tree or as the central element of garden compositions [12]. Owing to its drought hardiness and low maintenance cost, black mulberry is perfect for edible landscaping in changing environmental conditions [13]. While searching for appropriate tree species for urban habitats affected by climate change, Roloff et al. [14] suggested white mulberry as very suitable and black mulberry as suitable for novel plannings in cities. Investigating the profitability of food trees planted in urban public green areas, (providing the physiological, sociological, economic and aesthetic benefits), Lafontaine-Messier et al. [15] found black mulberry as a 'value' tree. In a Montreal case study, surveying public urban fruit trees including mulberries, Colinas et al. [16] showed that similarly to community gardens and food foraging, public fruit production could serve as a potentially cost-effective means of improving the resilience and pro-environmental behavior of communities. Suggesting species for edible landscaping in urban horticulture, Fetouh [17] listed white and black mulberry among species with multiple functions, such as for food, flavor, and ornamental appearance. Additionally, Poguberović et al. [18] suggested the usage of mulberry leaves as extracts for the preparation of environmentally friendly, non-toxic and low-cost adsorbents to remediate urban pollutants Ni(II) and Cu(II) from aqueous solutions, due to their efficiency as well as mulberry tree abundance and easiness to be found in Vojvodina. Although highly represented in both public and private green areas in Serbia, mulberries are predominantly preferred for fruits [19,20], mainly in private gardens.

Berry fruits have received great attention over the past decade since many studies have highlighted their ability to impact human health and reduce the risk of certain types of diseases [21] and the effect of aging. Berries provide significant health benefits because of their high levels of polyphenols, vitamins, minerals, and fiber [22,23]. Many studies revealed that mulberry is a good source of bioactive compounds such as carotenoids, alkaloids [1,24,25], phenolics, vitamins, fatty acids and minerals [7,26–29]. Antioxidant, antimicrobial and anticarcinogenic activities are mainly linked to the presence of phenolics [30].

The present research targets morphological traits of vegetative growth parameters, pomological and chemical evaluation of selected mulberry genotypes to determine the genotypes most suitable for ornamental gardening purposes, with one word coined as 'ornafruits'. Thus, the objective of the present study was to evaluate *Morus* species from the Northern region of Serbia and to identify the genotypes with both superior landscaping (ornamental) and pomological (functional food) characteristics.

2. Materials and Methods

2.1. Plant Material

More than 300 mulberry genotypes growing in comparable environmental conditions were visited, and preliminary tree and fruit characteristics were qualitatively scored in situ, by the researcher panel consisting of landscape architects, horticulturalists, pomologists and breeders. The dendrological analysis included qualitative parameters—tree vigor (low, semi or vigorous), tree habit (upright, spreading or dropping), rotten trunk and branches presence (present or absent), phytopathological and entomological damages (present or absent), vitality and decorative value. Scores for vitality and decorativeness were provided by panelists according to the five-level scale (1—very low, 2—low, 3—medium, 4—high and 5—very high). Mulberry genotypes of specific overall appearance and observed morphological properties (highly scored for interesting growth habit, absence of disease and pest symptoms, decorativeness and vitality) were pre-selected from their in situ locations. The core collection in this survey resulted in 15 genotypes (Table 1) selected from various locations in the province of Vojvodina, Serbia, situated mainly in small private rural front and backyards as well as both rural and urban ornamental front yard gardens (Table 1). One exception was the genotype JP2 selected from the public school front yard.

Table 1. Sampling locations in Northern Serbia and list of *Morus* species studied.

Number	Species	Genotype Code	Place of Collecting	Type of Green Area
1.	*M. alba*	BP 1/4	Bačka Palanka	Rural back-yard
2.	*M. alba*	BP 3/9	Bačka Palanka	Rural back-yard
3.	*M. alba*	DJ1	Novi Karlovci	Rural back-yard
4.	*M. alba*	DT1	Inđija	Urban front-yard
5.	*M. alba*	JP2	Inđija	School front-yard
6.	*M. alba*	MG1	Novi Karlovci	Rural back-yard
7.	*M. alba*	PB1	Novi Sad	Urban front-yard
8.	*M. alba*	ZD1	Inđija	Urban front-yard
9.	*M. alba*	ZP1	Novi Karlovci	Rural front-yard
10.	*M. alba*	ZP2	Novi Karlovci	Rural front-yard
11.	*M. nigra*	BPJ	Bačka Palanka	Rural back-yard
12.	*M. nigra*	MN1	Bačka Palanka	Rural back-yard
13.	*M. nigra*	SK1	Sremski Karlovci	Urban front-yard
14.	*M. nigra*	ZP3	Novi Karlovci	Rural front-yard
15.	*M. rubra*	MR1	Inđija	Urban front-yard

Edible mulberry germplasm genotypes from Northern Serbia were identified and classified into three species *M. alba*, *M. nigra* and *M. rubra*, of which the majority belonged to *M. alba*. Vegetative parts for analysis were sampled in June, after intensive vegetative growth completion. Research related to morphological and pomological traits was conducted at the Faculty of Agriculture in Novi Sad, while the chemical investigation of fruits and determination of the content of specific compounds were performed at the Institute for Medicinal Plants Research 'Dr Josif Pančić' in Belgrade. For this research, berries were picked at the biologically ripe stage, determined by the harvesting easiness (resistance of the fruit stem to the fruit pulling) as well as fruit softness. Harvest time was determined between the 10th and 15th of June, depending on the genotype. Since two genotypes—BPJ and SK1—proved to be male specimens, further fruit analysis was not performed, resulting in 13 genotypes pomologically described.

2.2. Morphometric Analyses of Vegetative Parts and Fruit

Vegetative samples were collected after intensive spring growth was finished and the final size was achieved. Fruits were collected in their full ripening stage and transported to the laboratory in a hand refrigerator. Vegetative and pomological characterization of genotypes was carried out according to the international FAO descriptor [31] as in Kadri et al. [32]. Measurement of appropriate parameters was conducted on 15 samples of each genotype. The following morphometric and pomological parameters were studied: annual branches length (cm), branch thickness (mm), internodes length (cm), leaf length (cm), leaf width (cm), leaf shape index, petiole length (cm) and width (mm), a mass of ten leaves (g), fruit mass (g), fruit width (mm) and height (mm), peduncle length (mm) and width (mm) and fruit shape index. In addition, dry matter content in fruits was determined (%). Petiole length (cm) and width (mm), fruit height and width (mm) as well as peduncle length (mm) and width (mm) were measured by a Mitutoyo digital caliper (accuracy of ±0.01 mm), while dry matter content (%) was determined using a handheld E-Line refractometer 'ATC Range'.

2.3. Chemical Analysis of Mulberry Fruits

The chemical composition of fruits from selected genotypes was also analyzed. Fruit samples were used to determine total soluble solids content (SSC), total acidity (TA), total sugar content, reducing sugar, sucrose, ascorbic acid, total anthocyanin content (TAC) and total phenolic contents (TPC). Soluble solids (SS) content was determined by a refractometer (Atago, pocket PAL-1. Kyoto, Japan). Titratable acidity (TA) was determined by titrating 10 g of berries with 0.1 N NaOH up to pH 7.0. Acidity was expressed as the percent of malic acid. An iodometric titration method was performed for the determination of ascorbic acid (AA), and the results were expressed as milligram ascorbic acid/100 g fresh mass. Total sugars (TS), inverted sugar (IS) and sucrose (S) were determined by the Luff–Schoorl method in %. The total phenolic content was estimated by Folin–Ciocalteu method with slight modifications [33]. Juice samples (10 g equivalent of fresh berry) were extracted with MeOH for 30 min on the ultrasonic bath and then filtered. Two hundred microliters of extract was added to 1 mL of 1:10 diluted Folin–Ciocalteu reagent. After 4 min, 800 µL of sodium carbonate (75 g/L) was added. After 2 h of incubation at room temperature, the absorbance at 765 nm was measured. Gallic acid (0–100 mg/L) was used for the calibration of a standard curve. The results were expressed as milligrams of gallic acid equivalents per 100 grams of fresh mass (mg GAE/100 g FW). Total anthocyanin content was investigated according to the procedure described in European Pharmacopoeia 9.0 (E.R 9.0) with slight modifications. Then, 95 mL of methanol was added to 10 g of berry juice and mechanically stirred for 30 min, and then filtered into a 100 mL volumetric flask. The filter was rinsed and diluted to 100 mL with methanol. A 50-fold dilution of this solution in a 0.1% v/v solution of hydrochloric acid in methanol was prepared. The absorbance of the solution was measured at 528 nm, using a 0.1% v/v solution of hydrochloric acid in methanol as the compensation liquid.

2.4. Statistical Analysis

The obtained data were statistically processed by the analysis of variance, using STATISTICA 14 software (Tibco, Palo Alto, CA, USA). The significance of differences between the mean values of investigated parameters was determined by Duncan's multiple range tests with the confidence of $p \leq 0.05$.

3. Results and Discussion

3.1. Vegetative Characterization of Investigated Mulberry Genotypes

Given the horticultural and phytonutrient traits of mulberries, it is important to examine morpho-chemical characteristics of selected genotypes, before their selection, registration and propagation. In our country, there are no varieties selected based on combined nutritional and horticultural values, nor are there varieties recommended for commercial orchard cultivation. Hence, the complete morphological and pomological characterization of selected genotypes may serve the purpose of selecting interesting varieties such as fruits or 'ornafruits' [11]. Meena et al. [34] suggested mulberry among others (karonda, custard apple, khejri, mulberry, etc.) as suitable for backyard or kitchen gardening. Following the mentioned, qualitative vegetative growth characteristics were observed to select genotypes with an overall attractive appearance (Table 2). Genotypes BP 3/9, DT1, ZP3 and MR1 have semi-vigorous growth, dropping growth habits, different leaf shapes (ovate, oval, cordate) and leaf color (from light to dark green), corresponding to ornamental mulberries. Semi-vigorous genotype ZD1 with a spreading tree and interesting palmate-lobed leaves were distinguished as a unique genotype for edible gardening or landscaping purposes.

Table 2. Tree and leaf qualitative characteristics of mulberry genotypes.

Genotype Code	Leaf Color	Leaf Shape	Tree Vigor	Growth Habit
BP 1/4	Dark green	Cordate	Semi	Spreading
BP 3/9	Dark green	Cordate	Vigorous	Dropping
DJ	Green	Ovate	Vigorous	Dropping
DT1	Green	Oval	Vigorous	Dropping
JP2	Green	Heteromorphic	Vigorous	Spreading
MG1	Green	Ovate	Semi	Spreading
PB1	Green	Oval	Vigorous	Spreading
ZD1	Light green	Palmately lobed	Semi	Spreading
ZP1	Dark green	Cordate	Semi	Spreading
ZP2	Green	Ovate	Vigorous	Spreading
BPJ	Light green	Cordate	Vigorous	Spreading
MN1	Dark green	Cordate	Vigorous	Spreading
SK1	Dark green	Cordate	Vigorous	Spreading
ZP3	Light green	Cordate	Vigorous	Dropping
MR1	Green	Cordate	Semi	Dropping

To provide a complete picture of the morphological properties of the collected material, vegetative parts were analyzed. According to the data, variations were noticed among and within genotypes for some of the traits (Table 3). The most vigorous annual shoot growth was detected in the ZP3 genotype (118.5 cm), followed by DT1 (108.2 cm), MR1 (101.8 cm) and ZP1 (100.5 cm) genotypes. Contrary, genotype DJ1 exhibited the lowest annual growth with only a 32.9 cm average length of the shoots. The coefficient of variation CV over 40% for annual branches clearly shows that there were variations among genotypes, and high standard deviation (SD) indicates variations within genotypes. When observed between species, *M. alba* genotypes had smaller mean values (65.2 cm) followed by *M. nigra* (74.4 cm), compared to one *M. rubra* (101.8 cm). The widest average branch thickness was determined for ZP1 (8.1 cm), followed by ZP2, MG1 and ZP1 with 5.5, 5.2 and 5.1 cm, respectively. The average internode length ranged from 2.8 cm (DJ1) to 8.3 cm (DT1), with an absolute maximum of 10.4 cm in ZP3 and an absolute minimum of 2.0 cm in DJ1. The highest average value for leaf length was determined for ZP1 (18.1 cm), followed by MG1 (17.9 cm), ZP2 (17.2 cm) and PB1 (16.4 cm). The widest average value for leaf width was determined for the same genotypes, ZP1 (11.1 cm), followed by MG1 (14.1 cm), ZP2 (12.4 cm), SK1

(11.8 cm) and PB1 (11.2 cm). Mean averages for leaf length and leaf width amounted to 14.1 and 10.9 cm (respectively), with an absolute maximum of 22.0 and 21.0 cm for leaf length and leaf width determined in genotype ZP1.

Table 3. Morphometric characteristics of branches and leaves sampled from studied genotypes.

Genotype Code	Annual Branches Length (cm)	Branch Thickness (mm)	Internodes Length (cm)	Leaf Length (cm)	Leaf Width (cm)	Leaf Shape Index (Length/Width Ratio)	Petiole Length (cm)	Petiole Width (mm)	Mass of Ten Leaves (g)
BPJ	$47.7 \pm 7.6^{hi*}$	4.1 ± 0.5^{e}	5.5 ± 1.1^{e}	13.6 ± 1.6^{cd}	10.4 ± 1.9^{efg}	1.3 ± 0.1^{bcd}	3.8 ± 0.4^{h}	3.0 ± 0.3^{b}	21.7 ± 3.7^{e}
BP 1/4	36.7 ± 2.8^{j}	3.6 ± 0.1^{fg}	4.2 ± 0.5^{f}	12.2 ± 0.7^{e}	10.0 ± 0.9^{fg}	1.2 ± 0.1^{def}	4.2 ± 0.4^{gh}	2.7 ± 0.3^{c}	17.4 ± 2.1^{fg}
BP 3/9	43.6 ± 4.5^{i}	3.9 ± 0.6^{ef}	3.3 ± 0.9^{g}	12.4 ± 1.8^{e}	9.1 ± 1.3^{hi}	1.3 ± 0.1^{abc}	4.8 ± 0.7^{e}	2.4 ± 0.4^{d}	17.1 ± 2.5^{fg}
DJ1	32.9 ± 5.1^{j}	3.5 ± 0.2^{g}	2.8 ± 0.6^{g}	11.8 ± 2.1^{e}	8.6 ± 0.5^{i}	1.4 ± 0.3^{abc}	4.1 ± 0.3^{gh}	2.2 ± 0.3^{f}	16.8 ± 2.0^{fg}
DT1	108.2 ± 5.7^{b}	4.9 ± 0.2^{c}	8.3 ± 1.1^{a}	14.6 ± 1.2^{c}	10.0 ± 0.8^{fgh}	1.5 ± 0.2^{a}	5.2 ± 0.5^{d}	2.1 ± 0.2^{f}	16.5 ± 1.0^{gh}
JP2	51.6 ± 3.7^{h}	2.7 ± 0.3^{h}	5.0 ± 0.7^{e}	14.0 ± 2.2^{c}	10.8 ± 1.1^{def}	1.4 ± 0.2^{cde}	4.2 ± 0.4^{gh}	2.0 ± 0.3^{f}	14.3 ± 2.2^{i}
MG1	76.4 ± 6.3^{e}	5.2 ± 0.6^{c}	6.4 ± 0.4^{d}	17.9 ± 1.2^{a}	14.1 ± 0.9^{b}	1.3 ± 0.1^{cde}	6.4 ± 0.6^{b}	2.8 ± 0.2^{bc}	34.9 ± 5.8^{b}
MN1	61.5 ± 6.5^{g}	4.1 ± 0.4^{e}	5.0 ± 0.5^{e}	12.7 ± 1.1^{de}	9.8 ± 1.1^{gh}	$1.3 \pm 0.1^{b-e}$	4.1 ± 0.3^{gh}	2.6 ± 0.3^{cd}	17.5 ± 2.0^{fg}
MR1	101.8 ± 10.4^{c}	4.4 ± 0.4^{d}	7.6 ± 1.3^{abc}	12.0 ± 1.7^{e}	9.9 ± 1.3^{fgh}	1.3 ± 0.1^{ef}	5.9 ± 0.9^{c}	1.8 ± 0.2^{g}	14.5 ± 2.5^{hi}
PB1	46.4 ± 6.6^{i}	3.9 ± 0.3^{ef}	4.9 ± 0.5^{e}	16.4 ± 1.4^{b}	11.2 ± 1.4^{de}	1.5 ± 0.2^{a}	4.3 ± 0.3^{fg}	2.5 ± 0.2^{d}	23.8 ± 2.0^{d}
SK1	69.8 ± 3.6^{f}	4.9 ± 0.5^{c}	8.2 ± 1.6^{ab}	12.8 ± 1.6^{de}	11.8 ± 1.4^{cd}	1.1 ± 0.1^{g}	7.3 ± 0.8^{a}	2.4 ± 0.6^{de}	17.2 ± 1.7^{fg}
ZD1	82.5 ± 0.3^{d}	4.5 ± 0.9^{d}	7.1 ± 1.5^{cd}	10.2 ± 1.0^{f}	9.0 ± 1.0^{hi}	1.1 ± 0.2^{fg}	4.7 ± 0.6^{ef}	1.8 ± 0.1^{g}	6.4 ± 0.2^{j}
ZP1	100.5 ± 10.7^{c}	8.1 ± 0.8^{a}	6.9 ± 0.9^{d}	18.1 ± 2.3^{a}	15.1 ± 2.7^{a}	1.2 ± 0.1^{ef}	7.0 ± 0.5^{a}	3.6 ± 0.6^{a}	40.1 ± 7.1^{a}
ZP2	73.1 ± 3.3^{ef}	5.5 ± 0.2^{b}	5.2 ± 1.0^{e}	17.2 ± 1.1^{ab}	12.4 ± 1.4^{c}	1.4 ± 0.2^{ab}	4.1 ± 0.2^{gh}	3.0 ± 0.4^{b}	28.8 ± 1.7^{c}
ZP3	118.5 ± 8.4^{a}	5.1 ± 0.0^{c}	7.6 ± 1.7^{bc}	12.9 ± 1.6^{de}	10.7 ± 1.4^{efg}	1.2 ± 0.1^{ef}	5.9 ± 0.5^{c}	2.2 ± 0.3^{ef}	18.9 ± 3.5^{f}
$\overline{x}$	69.4	4.6	5.7	14.1	10.9	1.3	5.0	2.5	20.8
A_{min}	30.0	2.2	2.0	8.1	7.0	0.8	3.0	1.1	6.2
A_{max}	128.4	9.2	10.4	22.0	21.0	2.7	9.0	5.1	51.4
S.D.	28.5	1.3	1.9	2.8	2.3	0.2	1.2	0.6	8.4
CV%	41.1	29.4	34.3	19.8	20.7	14.6	24.1	24.3	40.7

* Means designated with the same letter within a single column were not significantly different according to Duncan's multiple range tests ($p \leq 0.05$). A_{min}, absolute minimum; A_{max}, absolute maximum; S.D., standard deviation.

Ten leaf masses followed the same trend as determined for leaf length and width. The greatest average value for ten leaves mass was determined in ZP1 (40.1 g), followed by MG1 (34.9 cm), ZP2 (28.8 cm) and PB1 (23.8 cm). An absolute maximum of 51.4 g was determined for ZP1 and an absolute minimum of 6.2 g for ZD1. It was previously reported by Pentón et al. [35] that the genus *Morus* is known for variations of morphometric traits, which our study confirmed.

The diversity of studied genotypes is confirmed through most studied traits, and it is reflected in large differences in the mean values, a wide range between absolute minimum and absolute maximum values and a high coefficient of variation. Genetic variability among genotypes can be attributed to the heterozygote nature of genotypes [36]. Investigating the morphological variation of 110 *M. alba* genotypes, Farahani et al. [37] determined that most of the traits exhibited significant differences among the studied genotypes. On the contrary, the most stable parameter was the leaf shape index (quantitative ratio between leaf height and width) for which the standard deviation (SD) was 0.2, and the coefficient of variation (CV) was 14.6%. In general, leaf shape (Figure 1) as a qualitative characteristic is considered a stable taxonomic characteristic most frequently used for plant determination; thus, its stability is presumed to be high within the genotypes of the same species, previously confirmed for cherries [38] and sweet potato [39]. In mulberries, Lo Bianco and Mirabella [40] successfully used leaf morphometric descriptors, images and multivariate analysis for discriminating mulberry cultivars. Although mulberry leaves are heteromorphic, accompanied by qualitative characterization (margins incision, leaf blade smoothness, leaf blade shininess and similar), quantitate measurements can help in the determination of true-to-type genotypes. In a very recent study [41], high heritability (h^2) was recorded for mulberry leaf properties, ranging from 0.95 to 0.99.

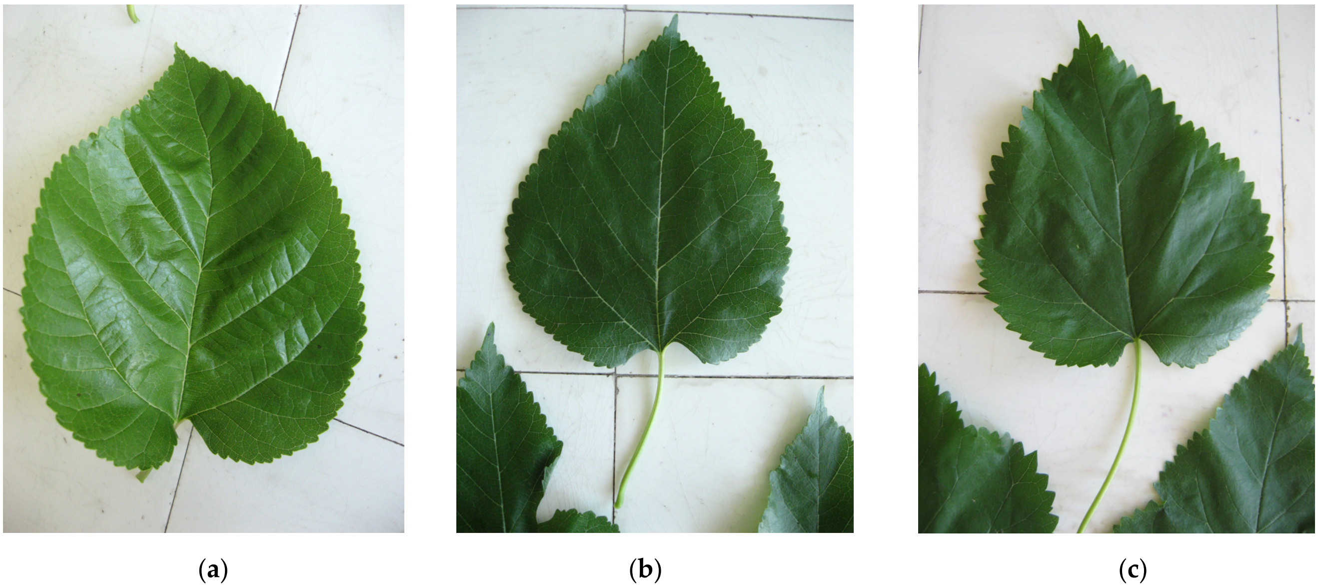

Figure 1. Leaf shape of white mulberry—DJ1 'ovate' (**a**), black mulberry—ZP3 'cordate' (**b**) and red mulberry—MR1 'cordate' (**c**).

3.2. Pomological Characterization of Investigated Mulberry Genotypes

Results obtained for the characterization of fruit mass, height and width, fruit shape index, peduncle length and width as well as dry matter content are presented in Table 4 and Figure 2. Fruit mass, as one of the most important agronomic traits and one of the main objectives of selection, significantly varied among genotypes, with a high coefficient of variation (43.7%). Previously, Özgen et al. [42] reported a lower coefficient of variation (CV) for fruit mass up to 26% of *M. alba*, *M. rubra* and *M. nigra* genotypes from Turkey, indicating a possible greater genetic distance of pertinent genotypes from Serbia. Fruit mass ranged from 1.4 g (DT1) to 6.1 g (DJ1), with an average of 3.5 g. An average value over 4.00 g was achieved in genotypes MG1, MR1, PB1 and BP1/4. The absolute maximum for fruit mass appeared among samples from genotype DJ and amounted to 8.3 g, while an absolute minimum of 1.1 g appeared among samples from genotype DT1. Our pomological results were comparable with previously provided data from Turkish [7,42] and Spanish [43] researchers with a remark that there were samples with fruit mass values (absolute minimum and absolute maximum) lower and higher than those previously reported ranges. Some of our genotypes even surpassed the results obtained in the most recent studies, including mulberry selections with 0.75–5.02 g [37,44] as well as the commercial cultivar 'Chinese Long' with 6.26 g [45]. Our results showed that fruits of *M. rubra* can reach a maximal 6.4 g, and an average of 4.9 g, corroborating the previous results of Ercisli and Orhan [7] and Özgen et al. [42], with the fruit mass of *M. alba*, *M. rubra* and *M. nigra* genotypes in the ranges of 2.14–4.37 g and 3.3–8.2 g, per a study, respectively. Sanchez et al. [43] reported that *M. nigra* genotypes had bigger fruits, while in the pertinent study, black mulberry achieved above-average results. Differences in results are not surprising considering genotypic and different environmental effects.

Table 4. Pomological traits of *Morus* sp. genotypes.

Genotype Code	Fruit Mass (g)	Fruit Height (mm)	Fruit Width (mm)	Fruit Shape Index	Peduncle Length (mm)	Peduncle Width (mm)	Dry Matter Content (%)
BP1/4	4.2 ± 1.0 c*	29.2 ± 3.3 b	15.1 ± 2.0 bc	2.0 ± 0.3 c	7.1 ± 1.2 f	1.4 ± 0.1 b	14.0 ± 1.1 ef
BP3/9	3.5 ± 0.7 d	25.8 ± 2.4 c	14.6 ± 1.0 c	1.8 ± 0.1 de	8.4 ± 1.1 e	1.0 ± 0.1 ef	16.9 ± 1.4 c
DJ1	6.1 ± 0.8 a	33.2 ± 2.1 a	17.6 ± 1.6 a	1.9 ± 0.1 cd	13.7 ± 1.6 a	1.3 ± 0.3 bcd	15.2 ± 0.9 de
DT1	1.4 ± 0.2 f	18.5 ± 1.6 e	10.2 ± 1.4 g	1.8 ± 0.2 cde	11.2 ± 1.3 c	0.7 ± 0.1 h	19.1 ± 1.5 b
JP2	1.9 ± 0.4 f	21.3 ± 2.2 d	12.0 ± 0.9 f	1.8 ± 0.2 de	7.1 ± 1.0 f	0.8 ± 0.2 gh	16.1 ± 1.2 cd
MG1	5.0 ± 0.8 b	34.8 ± 2.9 a	14.2 ± 1.4 cde	2.5 ± 0.2 a	12.6 ± 0.9 b	1.6 ± 0.2 a	13.0 ± 1.3 f
MN1	2.8 ± 0.9 e	24.7 ± 3.3 c	13.1 ± 2.1 e	1.9 ± 0.2 cd	9.9 ± 1.1 d	0.9 ± 0.2 fgh	11.7 ± 1.2 g
MR1	4.9 ± 0.9 b	29.5 ± 3.6 b	17.3 ± 2.5 a	1.7 ± 0.4 de	10.4 ± 1.1 cd	1.2 ± 0.2 cde	19.8 ± 2.4 b
PB1	4.2 ± 0.8 c	30.0 ± 2.7 b	16.0 ± 1.2 b	1.9 ± 0.2 cde	13.9 ± 0.9 a	1.3 ± 0.2 bc	14.8 ± 1.6 de
ZD1	2.7 ± 1.0 e	20.9 ± 4.0 d	13.3 ± 1.2 de	1.6 ± 0.2 f	5.3 ± 0.8 g	0.9 ± 0.2 fg	24.9 ± 1.5 a
ZP1	3.4 ± 0.6 d	24.4 ± 2.8 c	14.4 ± 0.9 cd	1.7 ± 0.2 ef	12.5 ± 0.9 b	1.1 ± 0.2 de	19.6 ± 1.9 b
ZP2	3.5 ± 0.7 d	29.5 ± 3.1 b	13.3 ± 1.4 de	2.2 ± 0.2 b	10.5 ± 0.9 cd	0.9 ± 0.2 fg	16.7 ± 1.3 c
ZP3	1.8 ± 0.3 f	20.0 ± 2.6 de	11.4 ± 0.9 f	1.7 ± 0.2 de	10.4 ± 0.9 cd	0.8 ± 0.2 gh	13.2 ± 1.1 f
Mean	3.5	26.2	14.0	1.9	10.2	1.1	16.5
Absolute minimum	1.1	14.8	8.4	1.1	2.1	0.3	11.2
Absolute maximum	8.3	38.9	21.3	3.0	20.3	2.2	28.2
St. Dev.	1.5	5.7	2.5	0.3	3.6	0.3	3.8
CV(%)	43.7	21.7	18.1	21.5	34.7	29.6	22.8

* Means designated with the same letter within a single column were not significantly different according to the Duncan's multiple range tests ($p \leq 0.05$).

(**a**) (**b**) (**c**)

Figure 2. Fruit (berry) appearance of white mulberry—DJ1 (**a**), black mulberry—ZP3 (**b**) and red mulberry—MR1 (**c**).

Considering the mean values of fruit height, genotypes DJ1 and MG1 were distinguished by 33.2 and 34.6 mm (respectively), again surpassing the results of previously mentioned fruit investigations [7,42,43]. The average for all genotypes' fruit width was 14.0 mm and ranged from 10.2 (DT1) to 17.6 mm (DJ1). An absolute maximum for a fruit width of 21.3 mm was noted within samples of genotype DT1. Özgen et al. [42] reported fruit height and width in the ranges of 23.6–35.0 and 16.1–21.1 mm, respectively, while the range for fruit height was 20.5–30.3 mm in *M. alba* and *M. nigra* [43]. Our *M. alba* genotypes had higher values of fruit height compared to the Spanish type. Significant variations among genotypes considering peduncle length and width were noted according to high coefficients of variation. Dry matter content ranged from 11.7% (MN1) to 24.9% (ZD1) with an average of 16.5%, while the absolute maximum was 28.2% (ZD1) and the minimum was 11.2% (MN1). These results are similar to Farahani et al. [37] who found a variation from 9.3–32%.

3.3. The Chemical Composition of Fruits Belonging to Selected Genotypes

Although fruit samples were collected and immediately stored in a hand refrigerator until reaching the laboratory, some perishable genotypes showed a fast decline and very weak transportability and thus were excluded from further analysis. The antioxidant activities of mulberry fruits and their link to the presence of phenolics have been investigated and confirmed by various researchers in previous studies [20,46,47]. As high phenolic content and high antioxidant activity increase the nutritive and phytomedicinal potentials of mulberry, it was important to examine the chemical value of the fruits from the selected genotypes. High values of fruit mass do not necessarily mean high content of bioactive compounds, and in that sense, it is not the only important trait to rely on when it comes to selection. Table 5 provides the results of the fruit chemical analysis for genotypes MN1, ZP3, DT1, MR1, ZP2, ZP1 and BP 3/9. Soluble solids content (SSC) in white mulberry genotypes ranged from 11.5 (MN1) to 15.8% (ZP3). The highest SSC (21.2%) was associated with purple fruits belonging to red mulberry genotype MR1. These results are in agreement with previously reported data [7,42], although some studies had shown that purple mulberry fruits had lower soluble solid content than white and black mulberry [7,26]. The variation of SSC in mulberry fruits could be a result of the heterozygote nature of genotypes and the effect on different environmental conditions where the genotypes were grown [36]. The highest values for the SSC besides MR1 were determined for DT1 and BP 3/9. Since genotypes MR1, DT1 and BP 3/9 also had a high amount of dry matter content 19.8%, 19.1%, and 16.9%, respectively (determined by hand refractometer), those could be recommended for processing.

Table 5. Chemical composition of investigated mulberry fruits.

Genotype Code	Soluble Solids Content (%)	Total Acidity (%)	Total Sugar Content (%)	Reducing Sugar Content (%)	Sucrose Content (%)	Ascorbic Acid (mg/%)	Total Anthocyanins Content (%)	Total phenolic Contents (mgGAE/100 g Fresh Mass)
MN1	11.5 ± 1.2 ^{e*}	0.40 ± 0.0 ^{a}	8.97 ± 1.1 ^{e}	6.81 ± 0.7 ^{f}	2.05 ± 0.2 ^{a}	35.20 ± 2.6 ^{c}	0.05 ± 0.01 ^{b}	147.84 ± 0.82 ^{c}
ZP3	15.8 ± 0.7 ^{c}	0.43 ± 0.1 ^{a}	12.08 ± 1.2 ^{d}	10.10 ± 0.9 ^{e}	1.88 ± 0.1 ^{b}	42.24 ± 3.0 ^{b}	0.10 ± 0.01 ^{a}	185.03 ± 0.96 ^{b}
DT1	18.5 ± 1.0 ^{b}	0.38 ± 0.1 ^{a}	15.20 ± 1.5 ^{b}	13.96 ± 0.8 ^{b}	1.18 ± 0.1 ^{c}	49.28 ± 1.8 ^{a}	0.12 ± 0.01 ^{a}	221.08 ± 0.61 ^{a}
MR1	21.2 ± 1.7 ^{a}	0.19 ± 0.0 ^{cd}	17.41 ± 1.5 ^{a}	16.30 ± 0.9 ^{a}	1.05 ± 0.1 ^{d}	42.24 ± 2.7 ^{b}	-	63.23 ± 1.28 ^{d}
ZP2	13.4 ± 1.1 ^{d}	0.27 ± 0.1 ^{b}	12.08 ± 1.0 ^{d}	10.14 ± 1.1 ^{e}	1.84 ± 0.1 ^{b}	31.68 ± 1.8 ^{d}	-	44.93 ± 0.51 ^{e}
ZP1	14.3 ± 1.3 ^{d}	0.21 ± 0.0 ^{bc}	13.20 ± 0.8 ^{c}	11.20 ± 0.8 ^{d}	1.90 ± 0.1 ^{b}	17.60 ± 0.9 ^{f}	-	34.87 ± 0.31 ^{e}
BP 3/9	15.8 ± 1.2 ^{c}	0.13 ± 0.0 ^{d}	14.41 ± 0.8 ^{b}	12.21 ± 1.0 ^{c}	2.09 ± 0.1 ^{a}	21.12 ± 1.5 ^{e}	-	16.51 ± 0.29 ^{f}

* Means designated with the same letter within a single column were not significantly different according to Duncan's multiple range tests ($p \leq 0.05$).

The total acidity (TA) ranged from 0.13 to 0.43%. TA of black mulberry genotypes was between 0.40 and 0.43%, whereas these values were between 0.13 and 0.38% in white mulberry genotypes. The TA of purple mulberry MR1 was 0.19%. Purple mulberry had lower acidity compared to the majority of the investigated black and white mulberry genotypes. Previous reports showed higher values of total acidity in other regions [1,7,26,30,42]. Considering SSC and acidity together, genotypes DT1 and ZP3 may be recommended for fresh fruit production since they have attractive dark-colored fruits in combination with high fruit SSC and TA, which gives them a pleasant taste.

The highest amount of total sugar content (TSC) was associated with the purple mulberry fruits of MR1 (17.41%). Furthermore, *M. alba* genotypes appear to have a higher amount of sugar (12.08%-15.20%) than *M. nigra* genotypes (8.97%-12.08%). A similar trend was also observed for reducing sugar. Elmacı and Altuğ [48] reported for *M. nigra* that sugar content ranged from 11.3% to 16.2%, which was comparable to our results, while Imran et al. [1] found a lower amount of sugar in mulberry fruits. The presence of important amounts of sugars in mulberries should encourage their use as natural sugar sources in different food recipes. Sucrose ranged from 1.05 (MR1) to 2.09% (BP 3/9). Sucrose is present in low amounts due to the state of maturity of the berries, as the concentration of sucrose decreases regularly in mulberry fruits as maturity progresses [43].

A proper equilibrium between organic acids and sugars is essential for the pleasing flavor of fruits. Organic acids have an important influence on taste because their presence reduces the sweetness of fruits, exposing their sourness. The content of ascorbic acid ranged from 17.60 (ZP1) to 49.28 mg/% (DT1) and was variable in genotypes within and among genotypes. Considerably different contents of ascorbic acid have been reported by various authors [7,36,42,49]. The differences between genotypes in terms of the content of ascorbic acid might be caused by genetic factors as well as ecological factors—temperature, light, humidity, etc. [49]. Genotypes DT1, MR1 and ZP3 are characterized by a great amount of ascorbic acid, which indicates their selective value. The mulberry fruit is consumed in both fresh and processed forms. Traditionally, mulberry fruits are used for making juices, syrup, compotes, jams and fruit brandy (‘rakija’). Fruits that are used for making fruit brandy and jams are supposed to satisfy some sensory properties in terms of taste and aroma intensity, and genotypes should be high yielding. Genotypes with very sweet fruits and strong aromas are preferred. Genotypes that fulfill most of these terms are DJ, DT, MR, ZD1 and ZP2.

Anthocyanin pigments in mulberry fruits have a dual role. They affect the coloration of fruits during the last ripening stage and are considered secondary metabolites with potential nutritional value. Anthocyanins are considered very good antioxidant agents with high activity, which is attributed to their peculiar structure [27]. Results showed that three black-colored fruits contained anthocyanins (TAC) in the range of 0.05–0.12%. These genotypes also showed higher amounts of total phenolic content (147.84–221.08 mg GAE/100 g fresh weight), several times higher than the content of phenolics found in the pink- and white-colored mulberries. Pink- and white-colored mulberry samples were characterized by the absence of anthocyanins, and total phenolic content (TPC) values ranged from 16.51 to 63.23 mg GAE/100 g fresh weight.

The variation of TPC in the fruits depends on many factors, such as the degree of maturity at harvest, genetic differences, and environmental conditions during fruit development. According to our results, the highest content of total phenolics was found in the black-colored white mulberry genotype DT1 (TPC = 221.08 mg GAE/100 g fresh mass), while the lowest TPC value was obtained for the white-colored white mulberry genotype BP 3/9 (TPC = 16.51 mg GAE/100 g fresh mass). A previous study on *M. alba* grown in Vojvodina, North Serbia, reported higher TPC (43.84 to 326.29 mg GAE/100 g frozen fruit) [20], while a study of *M. nigra* from Southeast Serbia found lower TPC (90.26–118.84 mg GAE/ 100 g fresh mass) [25] compared to the present study. The TPC of *M. nigra* selected from a natural population in the vicinity of Belgrade, Serbia, was 177.51 mg GAE/100 g frozen fruit [29]. When compared to other publications, the investigated genotypes contained a significantly lower amount of total phenolics in fruits [1,7,27,36,42]. Although, phenolic compounds could be considered the main bioactive compounds contributing to the antioxidant activity of mulberry fruits, our chemical analyses showed that the most promising genotypes were MR1 and DT1 combining high SSC, total sugar content and ascorbic acid content. Genotype DT1 also had the highest TPC, although it is characterized by a small fruit mass due to the vigorousness of its tree and high yielding.

Fruit properties (both appearance and nutritive value) are important when deciding which species or cultivars to apply for edible landscaping (or urban foraging) purposes. According to Al-Mayahi et al. [50], the top gardening motives in Oman were esthetic, shading, the joy of hobby, functional-food source, physical exercise and environmental protection.

Homan [51] stated that well-known examples of urban foraged plants are mulberries as well as blackberries, apples, acorns and sweet chestnuts. Quantifying the nutritive value of four common urban species (serviceberry, mulberry, apple and black walnut), Bunge et al. [52] noted that urban foraging is an under-explored aspect of the alternative functional food movement.

Getting the general public accustomed to edible ornamentals as well as their nutritional values might increase their usage. Haight [53] concluded that only half of the participants in their study knew that quince and mulberry are edible. Amani-Beni et al. [54] noted that the integration of fruit-bearing trees, including mulberries, in the Akbarieh garden in Iran was a functional-food urban gardening solution.

Besides rural gardens where traditionally vegetables, flowers, spices, aromatic and medicinal herbs, decorative dendrological species, fruit species and vines can be grown separately or in combination on a considerable share of land, urban gardening recently comes in a form of a small kitchen or even Nutri-garden. Nutri-gardens are advanced forms of kitchen gardens in which vegetables are grown along with fruit, herbs, spices and other useful plants such as medicinal plants as a supplementary source of food or small income [55]. With the proven content of reducing sugars, total anthocyanins- and phenolic-investigated mulberry genotypes can have added value to their decorativeness and can be a part of both rural and urban edible gardening.

4. Conclusions

Since mulberry fruits are a source of functional food with therapeutic and nutritional applications, breeding strategies and selection from the existing gene pool aim to distinguish genotypes with high ornamental and pomological quality. Genotypes BP 3/9, DT1, ZP3 and MR1 have semi-vigorous growth, dropping growth habits, different leaf shapes (ovate, oval, cordate) and leaf color (from light to dark green), corresponding to the ornamental aspects of mulberries. Combined with the pomological and chemical characterization, those genotypes can be recommended for edible gardening purposes due to both the aesthetic appearance and nutritive value of their fruits. Some selected mulberry genotypes showed promising and interesting physicochemical properties for both fresh consumption and processing. For instance, if the appropriateness for fresh consumption is based on 'appearance' (higher fruit mass and attractive color), the best genotypes would be BP 1/4, DJ1, MG and MR1. Considering SSC and acidity together, genotypes DT1 and ZP3 may also be recommended for fresh fruit production. However, if classification is based on high intensity of sweetness, the best cultivars would be DJ1, DT1, MR1, ZD1, and ZP1, while genotypes MR1, DT1 and BP 3/9, due to the high SSC and high amount of dry matter content, could be recommended for processing into juices, syrup, compotes, jams or fruit brandy. According to the fruit's chemical composition and functional food value, the most promising genotypes were MR1 and DT1 where DT1 distinguishes itself for the highest content of total phenolics and total anthocyanins. Besides chemically analyzed genotypes, ones with more perishable fruits should not be discarded, but further studies are recommended for home gardening and edible landscaping purposes where fruits would be used for fresh consumption in the immediate residence vicinity.

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