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Autochthonous Cherry Rootstock Germplasm in the Context of Sustainable Sweet Cherry Production

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Abstract: Sustainability of fruit production is becoming a necessity in the time of climate changes and severe environmental issues, including decreasing water availability and biodiversity loss. To overcome these difficulties in sweet cherry production, we aimed to investigate the autochthonous cherry germplasm as a source of adaptable, size-controlling and productive rootstocks. The performance of sweet cherry cultivar 'Summit' grafted on six rootstock candidates and 'Gisela 5' as a control has been assessed in semi-arid climate, in conditions without irrigation and pruning, and with minimal herbicides' application. The qualitative (anchorage, suckering, vitality), vegetative (trunk cross sectional area—TCSA, tree dimensions) and generative (potential and achieved yielding, fruit quality) characteristics were investigated. All candidates provided adequate anchorage while three candidates did not form suckers. Trees on 'Gisela 5' showed the lowest vitality. The scion TCSA in the fifth vegetation ranged from 16.7 to 47.2 cm^2, while tree height, crown width and depth were up to 293, 150 and 175 cm, respectively. In sixth vegetation, the yield reached 4.1 kg. The average fruit mass in the trial of 2020–2021 was 8 g, fruit width was up to 27.5 cm, while the dry matter content reached 19%. The study showed that with the proper rootstock selection, sweet cherries could achieve satisfactory growth and yield without harming the environment and with minimal orchard's maintenance practices. Within investigated autochthonous material, candidate PC_02_01/4 induced the best performance of 'Summit' cultivar.

Keywords: adaptability; fruit quality; growth; 'Oblačinska' sour cherry; *Prunus cerasus*; *Prunus fruticosa*; rootstock breeding; yielding

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1. Introduction

The 2030 Agenda for Sustainable Development [1] encompassed 17 Sustainable Development Goals (SDG) aiming to build the more sustainable and resilient world for the generations to come. With world population expected to reach over 9 billion in 2050 causing an enormous surge in food demand [2], the food sector's transformation plays an important role in the achievement of indispensable actions towards the sustainability. An increasing competition for land, water and energy, affects and will continue to influence the growers' ability to produce food [3]. In order to overcome arising difficulties, the new perspectives in context of food production are sought. In terms of sustainable fruit production, different tools could be utilized to shift the focus from mostly yield-oriented fruit growing to the joint achievement of both crop quality and natural resources' conservation, as stressed by Jemrić et al. [4]. These tools imply the use of adequate plant material (rootstocks, wild fruit species) as well as major adjustments in orchards' management regarding, inter alia, the use of pesticides and irrigation practices.

The intensifying climate change may affect the tree crops at multiple levels, which endure severe stresses due to the occurrence of extreme low and high temperatures, lower precipitation and its unforeseeable monthly distribution and reduced water availability [5,6]. The drought events have become more frequent and water availability for irrigation purposes is steadily declining [7,8]. Water used in agriculture accounts for 70%

of total world's consumption [8], and is of immense importance in arid and semi-arid climates, were irrigation represents essential technique for the production intensification and producers' economic sustainability [6]. The adoption of deficit irrigation and the use of non-conventional water resources in order to overcome the meager water availability, especially in case of water-scarce areas, have been studied [9].

Over the past few decades, rootstock breeding efforts for many fruit species have been directed toward the selection of rootstocks which induce low-vigor of grafted trees, enabling the establishment of high density orchards for commercial fruit growing purposes. The main advantage of dwarfing rootstocks for sweet cherries is their effect on precocious fruiting and productivity improvement, in addition to the reduction of costs and facilitated maintenance of fruit plantations [10]. With growing need for natural resources' sustainable exploitation, the role of roots and rootstocks is becoming even more evident. Rootstock selection could be understood as a tool for the sustainable intensification of fruit production, considering the adaptability to temperature variations and extremes, cold tolerance, winter hardiness, tolerance to drought, water-logging, efficient water utilization, adaptability to suboptimal soil conditions, as well as the resistance/tolerance to pests and diseases [11,12]. Flachowsky and Hanke [13] stated that in times of great biodiversity loss worldwide, the sustainability of fruit production could be achieved only through the preservation of a high number of different species and genotypes, since future desired plant's traits cannot be predicted. The answers to many unpredictable future scenarios can be found in the wide spectrum of unexploited genetic material, and it is of great importance to ensure that locally adapted germplasm is not lost due to the modern and improved varieties' integration into fruit production [3].

The Balkan Peninsula provides vast gene pool for breeding efforts in *Prunus* sp. [14]. The long-term cultivation of *Prunus cerasus* L. ecovar. 'Oblačinska' in different agro-ecological conditions and the utilization of different propagation methods over the years (vegetative and generative), resulted in a mixture of numerous genotypes within the same population [15]. As a wild relative of sour cherry, *Prunus fruticosa* Pall.—European ground cherry is also characterized by a high biodiversity within species [16]. Both species are proven to be very resistant to low temperatures and drought, in addition to the induction of low vigor in grafted scions [17,18]. Although the diversity of 'Oblačinska' sour cherry has been studied by a number of authors, its potential for rootstock breeding purposes has not been completely exploited [19]. 'Oblačinska' sour cherry has been used for decades as a dwarfing rootstock for sweet and sour cherry in Serbia but its germplasm conservation and utilization in breeding programs have never been of national interest [20]. On the other hand, the European ground cherry is only sporadically used as a cherry rootstock [17]. In the past decades, morphological, phenological, anatomical and pomological research of potential size-controlling rootstocks belonging to local populations of both studied species have been carried out [21–26], charting a path to further investigations. Various investigations of scion-rootstock interaction, exploring numerous rootstocks and sweet cherry cultivars worldwide, pointed out the unequivocal rootstock's influence on different vegetative and generative traits [27–30].

We hypothesized that in extensive, non-irrigated trial investigated *P. cerasus* and *P. fruticosa* rootstock genotypes could induce dwarfed growth and better performance of sweet cherry cultivar 'Summit' regarding yielding potential, yielding and fruit quality, when compared to control trees on 'Gisela 5' and one *Prunus mahaleb* L. semi-vigorous rootstock candidate. Thus, this study aimed to investigate autochthonous cherry genotypes as potentially size-controlling and adaptable rootstocks, which enable the production of high quality sweet cherries in semi-arid conditions.

2. Materials and Methods

2.1. The Site Conditions and Plant Material

The investigation was carried out at the experimental field of Faculty of Agriculture, the University of Novi Sad, located in Rimski Šančevi, Northern Serbia (45°20′ N;

19°50′ E, 80 m above sea level). This region experiences a continental climate, characterized with extremely warm summers and cold winters. During the five-year experimental period (years 2017–2021), an average annual temperature was 13 °C, with maximum daily temperatures reaching 41 °C, and winter temperatures drops to −23 °C. Annual precipitation sum varied from 573 mm in 2017 to 721 mm in 2019 (Table 1). The meteorological parameters were measured by on-field installed weather station Metos AG/CP/DD (Pessl Instruments, Weiz, Austria). The soil type in the trial is chernozem, with 0.1% of coarse sand, 38.98% of fine sand, 37.8% of silt and 23.12% of clay in its composition, as previously described by Ljubojević and Narandžić [31]. The determined pH KCl and pH H_2O valued 7.21 and 7.78, respectively, with the content of calcium-carbonate ($CaCO_3$) of 3.26%, total organic C content of 1.69% and total N content of 0.14%.

Table 1. Main meteorological parameters during the experimental period (2017–2021).

Year	Maximal Average Monthly Air Temperature (°C)	Minimal Average Monthly Air Temperature (°C)	Average Annual Air Temperature(°C)	Annual Precipitation Sum (mm)
2017	24.1	−5.3	12.2	573
2018	23.7	1.2	12.9	673
2019	23.7	−0.2	13.1	721
2020	23.2	0.01	12.5	614
2021	24.7	2.7	12.1	627

Studied plant material included five cherry rootstock candidates grafted with sweet cherry cultivar ‘Summit’. Cultivar ‘Summit’ has been chosen as an old, standard variety, characterized with vigorous growth, late fruiting phase entering and lower productivity compared to some other commercially grown novel cultivars. In that way, rootstock influence on the poorly performing variety in addition to size-controlling effect was tested and the major advantages of the investigated rootstocks were assessed. ‘Gisela 5’ and *P. mahaleb* candidate were used as control rootstocks. For each accession, the abbreviation indicating genus and species was determined. Thus, rootstock candidates belonging to *P. cerasus* ecovar. ‘Oblačinska’ are designated with PC, the abbreviation PF stands for *P. fruticosa* candidates, while *P. mahaleb* rootstock candidate is designated with PM. Origin of different rootstock candidates (localities in Serbia where initial selection of genotypes was performed and plant material for propagation was sampled) is indicated with different numbers in the accession names, complemented with tier numbers. Therefore, in PC candidates’ accession names, 02 stands for Udovice and 05 stands for Irig. Numbers 01, 02 and 04 in PF rootstock candidates’ accession names refer to localities Popovica, Bački Jarak and Vrdnik, while for PM candidate number 09 stands for Rimski Šančevi. After in situ characterization and collection of cuttings from mother trees, rootstock specimens were propagated by green, softwood cuttings, which enabled the establishment of new ex situ collection in 2015. Grafted trees were planted with planting distance of 4 × 2 m. The trial included five plants per scion-rootstock combination, with randomized rootstock design. All trees were planted in the same site conditions (same part of the orchard) and evaluated from the aspect of qualitative, vegetative and generative parameters. In order to allow plants’ unaltered growth and evaluate the rootstock influence solely, pruning was not performed, in addition to the absence of irrigation and minimal usage of herbicides. The weeds in the rows were mowed occasionally to provide access to the trees, while the space between the rows was treated with the herbicide Sirius (Chemical Agrosava, Beograd, Serbia; dose 5 l/ha). The frost protection was not implemented, despite the terrain’s exposition to strong winds which occur during the colder months. The soil was not fertilized during the experiment.

2.2. Qualitive Traits' Assessment

During the four-year period 2017–2020, qualitative characteristics regarding adaptability, convenience and production quality of rootstock candidates were observed: anchorage, suckering tendency, as well as vitality of tested plants in conditions without irrigation. Anchorage was evaluated on a 1–5 score scale (1—very weak, 2—weak, 3—good, 4—very good, 5—excellent), while suckering tendency was assessed as: 0—absent, 1—low, 2—medium, 3—high. Vitality of plants was described on a 1–3 score scale, where values were represented as: 1—poor vitality, 2—good vitality, 3—excellent vitality.

2.3. Vegetative Growth Parameters' Measurement

From 2017 to 2020, trunk diameters (cm) of the rootstock, the graft union and the scion were measured with digital caliper (accuracy of ±0.01 mm). Measurements were conducted at the end of the vegetative period, in November. Trunk diameter of rootstock was measured 5 cm under the graft union, while measurement of scion trunk diameter was performed above the graft union, at the same distance. In the case of uneven radial trunk growth and some abnormalities formed on the trunk, the diameter was measured 5–10 cm from the graft union to avoid large deviations from the real measures. The trunk cross-sectional areas (TSCA, cm^2) of the rootstock, the graft union and the scion were calculated according to the following formula (Equation (1)):

$$\text{TSCA} = r^2 \times \pi \quad (1)$$

where r represents the radius of the trunk, assuming that the cross-section of trunk was a perfect circle.

In the same four-year period, tree height (cm), crown width (cm) and crown depth (cm) were measured for each tree, during dormancy period in November. The crown width was measured parallel to the row orientation, while the crown depth was measured at the 90° angle in relation to the row orientation, i.e., perpendicularly to the row line.

2.4. Evaluation of Yielding Potential, Achieved Yield and Fruit Quality

During the three-year period (2019–2021), the number and distribution of vegetative and generative elements on one- and two-year-old branches have been assessed, in February before the vegetative period has started. The bud distribution was assessed on 15 two-year-old branches and 15 one-year-old branches per scion-rootstock combination (3 regular shoots per 5 replicate plants per combination). Two-year-old branches were divided into three parts—the basal, the middle and the apical part. The number of nodes with no bud (blind buds), vegetative buds, fruit spurs and lateral branches was recorded on each part of the branch. Concerning the one-year-old branches, the number of vegetative and flower buds was assessed. The yielding potential was reflected through the number and distribution of fruit spurs and flower buds.

The fruit abundance per m^3 of crown volume was descriptively evaluated prior to the harvest in two consecutive years 2020–2021, using a wooden cube frame with a volume of 1 m^3. The score scale from 0 to 5 was used, where value 0 represents a total absence of fruits, while maximal value 5 represents abundant fruiting. After fruit abundance's visual assessment, all fruits from three replicate plants per scion-rootstock combination were harvested and yield (kg) per tree was calculated. In both 2020 and 2021, 25 fruits per scion-rootstock combination were measured. The fruit mass and stone mass (both in g) were measured using Kern electronic weighing scale, while fruit height, width and thickness (all in mm) were determined using a digital caliper (accuracy of ±0.01 mm), as described by López-Ortega et al. [32]. A mesocarp ratio (%) was calculated from determined fruit and stone mass for each sample. The soluble solids content (SSC, %) was measured by a handheld E-Line Refractometer 'ATC Range'. The petiole length (mm) for each fruit was assessed by a digital caliper. An organoleptic characterization was performed in accordance with UPOV descriptor (The International Union for the Protection of New

Varieties of Plants), by an expert panel consisting of eight members. The fruit skin and mesocarp color were evaluated, as well as a fruit taste, including acidity and sweetness level. The acidity levels were assessed as: 1—low, 2—medium, 3—high; while the sweetness was evaluated as: 3—low, 5—medium, 7—high.

2.5. Statistical Analysis

The obtained data were processed using Statistica 14 software (Tibco, Palo Alto, CA, USA). The experimental data obtained during the consecutive years were subjected to statistical analysis using Fisher's factorial analysis of variance—ANOVA to determine the influence of studied rootstock candidates on vegetative and generative features of grafted sweet cherry cultivar. The significance of differences between mean values of investigated parameters was determined by Duncan's multiple range tests with the confidence of $p \leq 0.05$.

3. Results

3.1. Qualitative Evaluation and Vegetative Growth of Grafted Sweet Cherry Trees

The qualitative characteristics of 'Summit' sweet cherry trees regarding rootstock candidate used for grafting, differed perceptibly within and between species (Table 2). The anchorage strength varied from good on PF_02_16 to excellent on PM_09_01 and 'Gisela 5'. Suckering tendency was the highest on rootstock candidates PF_01_01 and PF_02_16, while trees on PC_05_04, PF_04_09 and PM_09_01 showed complete absence of suckers during the experimental period, same as a 'Gisela 5'. All rootstock candidates were characterized with good vitality in conditions without irrigation and pesticides' application, unlike the trees on 'Gisela 5' which showed more pronounced symptoms of water stress (flagging and/or cupped leaves during the drought events).

Table 2. Qualitative traits of investigated trees grafted on different rootstock candidates.

Rootstock Candidate	Anchorage	Suckering	Vitality
PC_02_01/4	Very good	Low	Good
PC_05_04	Very good	Absent	Good
PF_01_01	Very good	High	Good
PF_02_16	Good	High	Good
PF_04_09	Very good	Absent	Good
PM_09_01	Excellent	Absent	Good
'Gisela 5'	Excellent	Absent	Poor

Trunk cross-sectional area (TCSA) of each graft part showed significant differences among trees on different rootstock candidates and 'Gisela 5' in 2017–2020 (Table 3). The TCSA of the rootstock varied from 3.5 to 11.7 cm^2 at the beginning of the experimental period, while in 2020 (the fifth vegetation) values ranged from 18.9 to 39.4 cm^2, with the highest values on PM_09_01 and 'Gisela 5'. The TCSA of the graft union in 2017 was within range of 5.5–14.4 cm^2 on rootstock candidates, which was significantly lower than in combination with 'Gisela 5' (21.9 cm^2). In 2020 the TCSA of the graft union varied from 27.4 cm^2 on PF_01_01 to 67.4 cm^2 on PC_02_01/4. The scion TCSA in 2017 reached 3.5 cm^2 on PF rootstock candidates, while the highest mean values were determined on PC_02_01/4 and 'Gisela 5' (9.5 cm^2 each). In the last experimental year, the TCSA of the scion again was the lowest on PF candidates (16.7–22.6 cm^2), while scions on PC candidates and 'Gisela 5' formed the group of same statistical significance, with values ranging from 35.8 to 47.2 cm^2. The same pattern was observed in all four consecutive years, for each graft segment, where the trees on PF rootstock candidates fell within the statistical group with the lowest and trees on PC rootstock candidates with the highest TCSA values.

Table 3. Trunk cross-sectional areas (TCSA) of grafted sweet cherry trees in four consecutive years.

Rootstock Candidate	TCSA of the Rootstock (cm^2)				TCSA of the Graft Union (cm^2)				TCSA of the Scion (cm^2)			
	2017	2018	2019	2020	2017	2018	2019	2020	2017	2018	2019	2020
PC_02_01/4	9.8 ^{a} *	17.4 ^{ab}	29.2 ^{ab}	32.8 ^{ab}	14.4 ^{b}	31.3 ^{ab}	50.7 ^{a}	67.4 ^{a}	9.5 ^{a}	22.4 ^{a}	35.4 ^{a}	47.2 ^{a}
PC_05_04	11.7 ^{a}	15.8 ^{ab}	23.0 ^{ab}	27.7 ^{ab}	13.3 ^{b}	29.2 ^{ab}	43.8 ^{a}	54.3 ^{b}	8.3 ^{a}	20.5 ^{a}	31.5 ^{ab}	35.8 ^{abc}
PF_01_01	4.5 ^{b}	9.8 ^{bc}	11.4 ^{c}	19.1 ^{b}	7.5 ^{c}	14.0 ^{c}	17.2 ^{b}	27.4 ^{d}	3.5 ^{b}	7.3 ^{b}	13.9 ^{c}	21.4 ^{d}
PF_02_16	4.6 ^{b}	10.4 ^{bc}	19.6 ^{bc}	31.6 ^{ab}	7.1 ^{c}	14.8 ^{c}	24.3 ^{b}	42.2 ^{bc}	3.1 ^{b}	9.6 ^{b}	15.2 ^{c}	16.7 ^{d}
PF_04_09	3.5 ^{b}	6.9 ^{c}	10.2 ^{c}	18.9 ^{b}	5.5 ^{c}	16.0 ^{c}	25.3 ^{b}	40.4 ^{c}	3.1 ^{b}	7.4 ^{b}	14.2 ^{c}	22.6 ^{cd}
PM_09_01	10.3 ^{a}	18.8 ^{a}	31.0 ^{a}	39.4 ^{a}	10.7 ^{bc}	25.3 ^{b}	43.0 ^{a}	52.3 ^{bc}	5.2 ^{b}	12.1 ^{b}	22.5 ^{bc}	28.1 ^{bcd}
'Gisela 5'	11.2 ^{a}	21.7 ^{a}	32.5 ^{a}	39.4 ^{a}	21.9 ^{a}	35.4 ^{a}	51.7 ^{a}	66.6 ^{a}	9.5 ^{a}	18.3 ^{a}	28.1 ^{ab}	38.1 ^{ab}

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

Data on sweet cherry trees' dimensions (Table 4) showed significant differences. In the first experimental year, tree height ranged from 135 to 216 cm, with the highest average value among trees on rootstocks candidates observed on PC_02_01/4 (196 cm). In general, trees on PC rootstock candidates were higher than those on PF and PM candidates, from whom only combination 'Summit'- PF_01_01 fell into the same statistical group as trees on PC candidates and 'Gisela 5'. Thus, in 2020 trees on both PC rootstock candidates, PF_01_01 and 'Gisela 5' reached above 290 cm in average height. The lowest height in 2020 was measured on PF_02_16 (220 cm), while average value for trees on PM candidate did not exceed 250 cm. Regarding crown width in 2017–2018, the highest values were determined on PC rootstock candidates, reaching up to 136 cm in 2018. In the two following years, crown with the highest width belonged to the trees grafted on both PC candidates and 'Gisela 5' (more than 130 cm). The lowest width in 2020 was recorded on PF_02_16 (105 cm). Crown depth values were within range of 50–95 cm in 2017, with significantly different values between trees on PC and PF rootstock candidates—the highest values belonging to the trees on PC_02_01/4 and PC_05_04. In the next three years, values measured on PF_01_01 were closer to the trees on PC candidates. The highest crown depth in 2020 was determined on PC_02_01/4—175 cm, while the lowest average value belonged to the trees on PF_04_09—115 cm.

Table 4. Dimensions of investigated trees grafted on different rootstock candidates during 2017–2020.

Rootstock Candidate	Tree Height (cm)				Crown Width (cm)				Crown Depth (cm)			
	2017	2018	2019	2020	2017	2018	2019	2020	2017	2018	2019	2020
PC_02_01/4	196 ^{a} *	235 ^{a}	273 ^{a}	293 ^{a}	101 ^{a}	136 ^{a}	145 ^{a}	150 ^{a}	95 ^{a}	140 ^{a}	164 ^{a}	175 ^{a}
PC_05_04	184 ^{ab}	230 ^{ab}	269 ^{a}	293 ^{a}	85 ^{ab}	128 ^{a}	130 ^{ab}	138 ^{ab}	84 ^{ab}	125 ^{ab}	140 ^{a}	153 ^{abc}
PF_01_01	160 ^{bc}	200 ^{bc}	265 ^{a}	290 ^{a}	60 ^{cd}	100 ^{bc}	110 ^{bc}	115 ^{bc}	60 ^{cd}	110 ^{b}	140 ^{a}	155 ^{abc}
PF_02_16	140 ^{c}	155 ^{d}	175 ^{b}	220 ^{c}	50 ^{d}	70 ^{d}	95 ^{c}	105 ^{c}	50 ^{d}	75 ^{c}	110 ^{b}	130 ^{bcd}
PF_04_09	135 ^{c}	170 ^{cd}	210 ^{b}	255 ^{b}	50 ^{d}	90 ^{cd}	110 ^{bc}	125 ^{abc}	55 ^{d}	80 ^{c}	100 ^{b}	115 ^{d}
PM_09_01	137 ^{c}	162 ^{d}	212 ^{b}	243 ^{bc}	64 ^{cd}	86 ^{cd}	103 ^{c}	116 ^{bc}	62 ^{cd}	83 ^{c}	108 ^{b}	123 ^{cd}
'Gisela 5'	216 ^{a}	248 ^{a}	269 ^{a}	293 ^{a}	79 ^{bc}	117 ^{ab}	132 ^{a}	140 ^{ab}	76 ^{bc}	131 ^{a}	139 ^{a}	157 ^{ab}

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

3.2. Bud Number and Distribution (Yielding Potential), Achieved Yielding and Fruit Characteristics of 'Summit' Cultivar

The number and distribution of vegetative and generative elements along the branches of 'Summit' trees during the three-year period varied significantly among scion-rootstock combinations and among vegetation periods (Figure 1). In general, at the basal part of a two-year-old branch, the nodes with no bud and vegetative buds were mostly located, while at the middle part the shift from nodes with no bud and vegetative buds' production to the fruit spurs' production was observed from 2019 to 2021. The apical part was characterized with the highest number of fruit spurs during all three experimental years, followed with the presence of lateral branches.

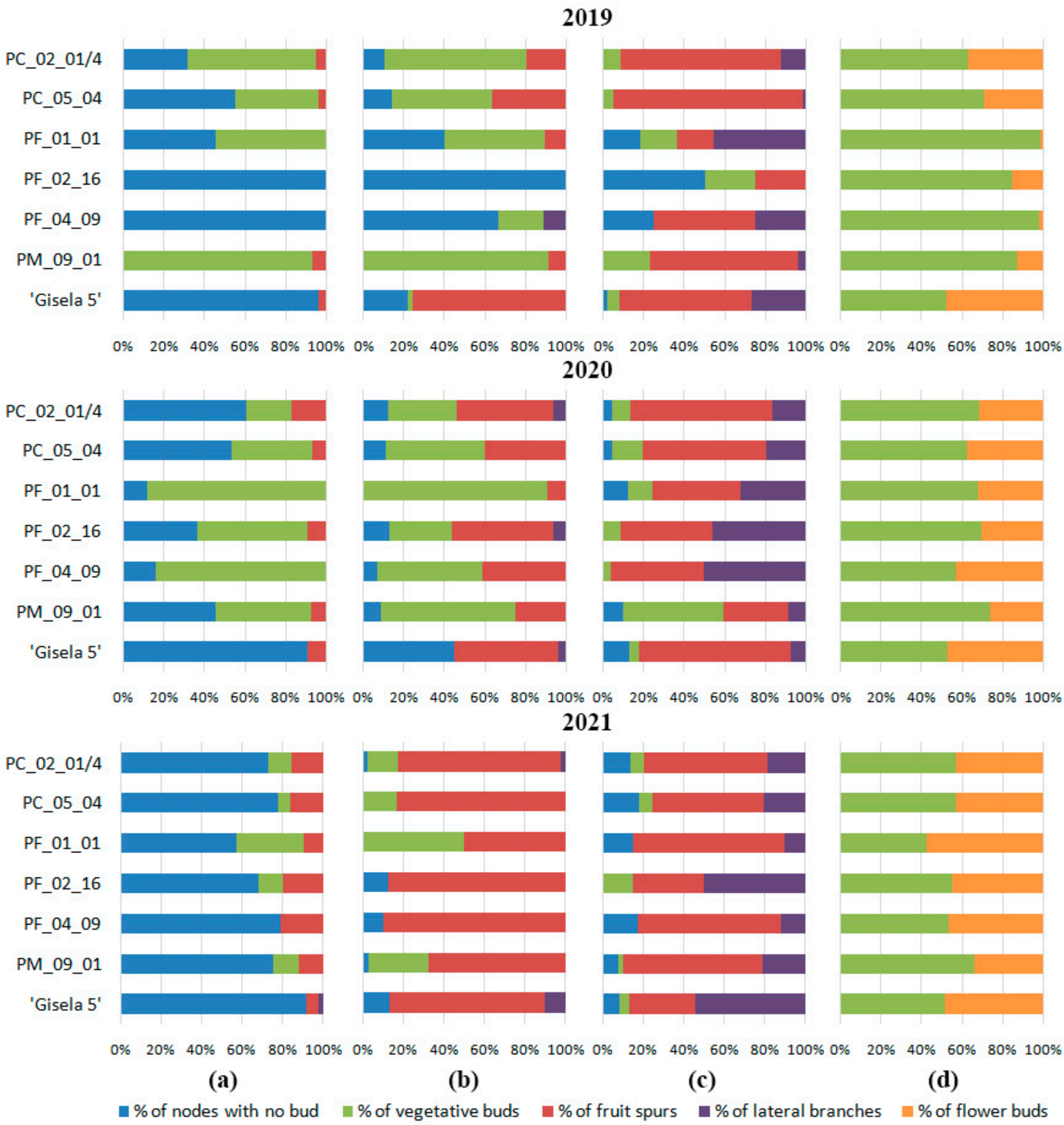

Figure 1. Bud distribution in three consecutive years at: (**a**) the basal part of a two-year-old branch; (**b**) the middle part of a two-year-old branch; (**c**) the apical part of a two-year-old branch; (**d**) the one-year-old branch.

In 2019 (the fourth vegetation) trees on PC rootstock candidates were characterized with 16.8% of nodes with no bud, 36.78% of vegetative buds, and the highest share of fruit spurs—43.9%, while the average share of lateral branches was low (2.5%). The trees on PF rootstock candidates had notably higher share of nodes with no bud—62.5% in average, with 19.6%, 9.2% and 8.7% of vegetative buds, fruit spurs and lateral branches along two-year old branches, respectively. The nodes with no bud were not recorded on plants grafted on PM candidate, which were characterized with the highest share of vegetative buds—59.8%, followed with 38.5% of fruit spurs and only 1.6% of lateral branches. The trees on 'Gisela 5' had almost equal share of nodes with no bud and fruit spurs—about 44%, and the lowest percentage of vegetative buds among all investigated trees—2.7%. Along the one-year old branches, the highest share of vegetative buds was recorded on PF candidates (93.8% in average), followed with trees on PM candidate—87.6%. Trees on PC candidates had about 67% of vegetative and 33% of flower buds, while trees on 'Gisela 5' had even share of both studied elements.

In 2020, the production of vegetative buds on PC rootstock candidates decreased approximately by 10%, combined with the slightly lower share of fruit spurs in comparison to the previous year—about 40%. The share of nodes with no bud and lateral branches concurrently increased. Trees on PF rootstock candidates showed a significant decrease in the share of nodes with no bud, which fell under 11%, in average. The percentage of vegetative buds, fruit spurs and lateral branches increased, with recorded average values of 46.3%, 27.4% and 15.8%, respectively. On PM candidate, the share of nodes with no bud increased by 23%, while the percentages of vegetative buds and fruit spurs were 52.9% and 21.2%, respectively. The bud distribution along branches on 'Summit'-'Gisela 5' trees remained almost the same as in the previous year, with a slight increase in the percentage of nodes with no bud and a decrease in the number of lateral branches (about 5% change). In average, about 65% of vegetative buds and 35% of fruit spurs were recorded along one-year branches on both PC and PF candidates. The highest share of vegetative buds was found on PM rootstock candidate—74.2%, while trees on 'Gisela 5' had the highest percentage of flower buds—47.1%.

According to the average group values (per rootstock species), the distribution of buds in the sixth vegetation (2021) showed similar share of both vegetative and generative elements on PC, PF and PM rootstock candidates. However, the share of nodes with no bud on rootstock candidates ranged from 27.3% on PF_01_01 to 41.3% on PF_04_09, the percentage of vegetative buds varied from none on PF_04_09 to 25.4% on PF_01_01, while the fruit spurs were present with 42.6% on PF_02_16 to 54.3% on PF_04_09. Compared to the trees on 'Gisela 5', almost all rootstock candidates induced the lower share of nodes with no buds and the higher share of vegetative buds along two-year old branches (41% and 1.6% on 'Gisela 5', respectively), with the exception of PF_04_09. With 33.6%, trees on 'Gisela 5' had notably lower share of fruit spurs in comparison to trees on investigated rootstock candidates. Only trees on 'Gisela 5' and PF_02_16 were characterized with more than 10% of lateral branches. Along the one-year branches, the highest share of vegetative buds was found on PM candidate—66.1%, while 57.4% of fruit spurs was recorded on PF_01_01.

The average yield per tree (Figure 2) in 2020 ranged from 0.1 kg on PF_02_16 to 3.6 kg on PC_02_01/4. Both PC rootstock candidates induced the higher yield in fifth vegetation in comparison to 'Gisela 5', which yielded 0.8 kg per tree. In the following year, harvested fruits per tree on 'Gisela 5' weighed 1.8 kg, while the highest yield was obtained on PC_02_01/4 (4.1 kg) and PF_04_09 (2.1 kg). Accordingly, the visually assessed fruit abundance on PC_02_01/4 (Table 5) was the highest among studied scion-rootstock combinations, reaching score 4 in both years. The lowest fruit abundance in 2019 was recorded on PF_02_16 and PM_09_01 (score 0.5), with only few fruits per tree produced. In the next vegetation, very low fruit abundance was observed on PC_05_04 and PM_09_01, scoring 1 on the fruit abundance's scale, which was in accordance with the yield measurements.

The fruit characteristics showed significant differences among scion-rootstock combinations (Table 6). The average fruit mass on PC, PF and PM candidates in 2020 was 7.7 g, 8.7 g and 7.2 g, respectively, while fruits harvested from trees on 'Gisela 5' weighed 8.8 g. In addition to trees on 'Gisela 5', fruits with average mass above 8 g and fruit width above 26 mm in the same year, were harvested on PC_02_01/4, PF_02_16 and PF_04_09. In the next year, the average fruit mass achieved on almost all rootstock candidates and 'Gisela 5' exceeded 7.5 g, followed with the fruit width above 25 mm. The exception were fruits on PF_04_09 and PM_09_01, with mass about 7 g and width below 25 mm. Fruit height reached 26.2 mm in 2020 and 24.2 mm in 2021, while fruit thickness valued up to 22.2 mm in 2020 and 21.4 mm in 2021. Mesocarp ratio in fifth vegetation was the highest on PC_05_04 and PF_01_01 (above 95%), while the fruits of trees grafted on 'Gisela 5' had the lowest value of 93.9%. In 2021 mesocarp ratio ranged from 94.5% on PC_05_04 and PF_02_16 to 95.9% on PC_02_01/4.

Table 5. Fruit abundance and taste of investigated fruits achieved on different rootstock candidates.

Rootstock Candidate	Fruit Abundance per m^3 *		Taste	
	2020	2021	Acidity	Sweetness
PC_02_01/4	4	4	medium	medium
PC_05_04	2.5	1	medium	medium
PF_01_01	2	2.5	low	medium
PF_02_16	0.5	3.5	low	low
PF_04_09	2	4	low	medium
PM_09_01	0.5	1	low	medium
'Gisela 5'	2	3	medium	low

* Fruit abundance: score scale 0–5, 0—no fruits, 5—abundant fruiting.

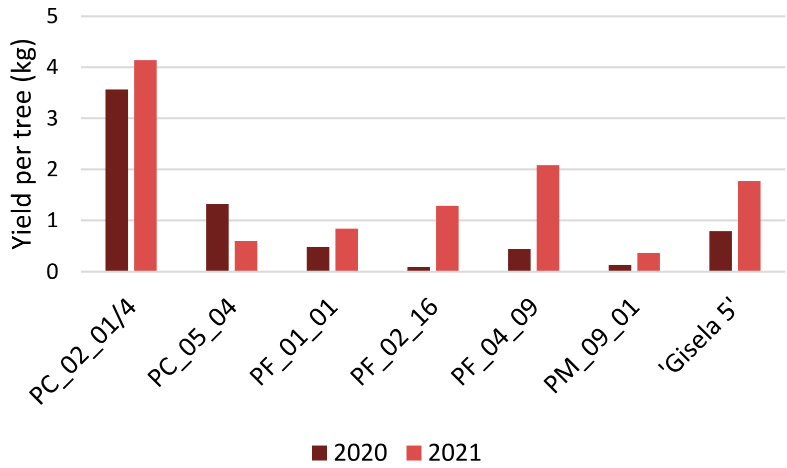

Figure 2. Yield per tree (kg) of seven scion-rootstock combinations in fifth and sixth vegetation period.

Table 6. Fruit characteristics of 'Summit' cultivar in two-year period (2020–2021).

Rootstock Candidate	Fruit Mass (g)	Fruit Height (mm)	Fruit Width (mm)	Fruit Thickness (mm)	Mesocarp Ratio (%)	Soluble Solids Content (%)	Petiole Length (mm)
2020							
PC_02_01/4	8.0 ± 0.8 ^{bc} *	24.7 ± 1.1 ^{bcd}	26.1 ± 0.8 ^{bc}	21.2 ± 0.6 ^{bc}	94.9 ± 0.5 ^{a}	15.5 ± 1.2 ^{b}	33.1 ± 2.4 ^{b}
PC_05_04	7.5 ± 0.8 ^{c}	24.4 ± 0.9 ^{cd}	25.2 ± 1.1 ^{cd}	20.7 ± 0.7 ^{cd}	95.0 ± 0.9 ^{a}	16.4 ± 1.4 ^{ab}	32.5 ± 3.2 ^{bc}
PF_01_01	7.9 ± 0.6 ^{bc}	25.4 ± 0.7 ^{abc}	25.9 ± 0.8 ^{bc}	21.3 ± 1.2 ^{abc}	95.4 ± 0.5 ^{a}	15.7 ± 0.9 ^{b}	29.5 ± 1.6 ^{c}
PF_02_16	9.1 ± 1.3 ^{a}	26.2 ± 1.6 ^{a}	27.5 ± 1.5 ^{a}	22.2 ± 0.8 ^{a}	94.1 ± 0.7 ^{bc}	14.0 ± 0.5 ^{c}	34.5 ± 5.3 ^{ab}
PF_04_09	9.2 ± 0.6 ^{a}	25.9 ± 0.8 ^{ab}	27.4 ± 0.8 ^{a}	22.1 ± 0.5 ^{ab}	94.8 ± 0.4 ^{ab}	15.2 ± 0.8 ^{b}	31.1 ± 1.7 ^{bc}
PM_09_01	7.2 ± 0.9 ^{c}	24.0 ± 1.9 ^{d}	24.5 ± 0.9 ^{d}	19.9 ± 0.5 ^{d}	94.8 ± 0.0 ^{ab}	17.4 ± 1.2 ^{a}	29.5 ± 7.8 ^{c}
'Gisela 5'	8.8 ± 1.1 ^{ab}	25.0 ± 1.4 $^{a-d}$	26.8 ± 1.3 ^{ab}	21.7 ± 1.0 ^{abc}	93.9 ± 0.6 ^{c}	15.3 ± 0.7 ^{b}	36.8 ± 3.1 ^{a}
2021							
PC_02_01/4	7.7 ± 1.0 ^{a}	23.7 ± 1.0 ^{ab}	25.3 ± 1.3 ^{ab}	20.8 ± 1.0 ^{ab}	95.9 ± 0.3 ^{a}	18.3 ± 0.8 ^{b}	34.3 ± 2.3 ^{b}
PC_05_04	7.7 ± 0.6 ^{a}	23.7 ± 0.7 ^{ab}	25.4 ± 0.9 ^{ab}	21.4 ± 0.6 ^{ab}	94.5 ± 1.0 ^{c}	19.0 ± 0.9 ^{a}	39.3 ± 3.7 ^{a}
PF_01_01	7.9 ± 0.7 ^{a}	24.0 ± 0.8 ^{ab}	25.8 ± 0.9 ^{a}	21.4 ± 1.8 ^{a}	95.4 ± 0.6 ^{ab}	15.8 ± 1.0 ^{cd}	35.7 ± 3.4 ^{b}
PF_02_16	7.5 ± 0.7 ^{ab}	24.2 ± 0.7 ^{a}	25.6 ± 1.0 ^{ab}	20.7 ± 0.6 ^{abc}	94.5 ± 0.6 ^{c}	15.2 ± 0.9 ^{de}	37.3 ± 3.5 ^{ab}
PF_04_09	7.1 ± 0.7 ^{bc}	23.3 ± 0.6 ^{b}	24.8 ± 1.0 ^{bc}	20.0 ± 0.7 ^{cd}	94.9 ± 0.6 ^{bc}	16.0 ± 1.1 ^{c}	34.9 ± 3.4 ^{b}
PM_09_01	6.9 ± 0.6 ^{c}	23.5 ± 0.8 ^{ab}	24.4 ± 0.7 ^{c}	19.7 ± 0.7 ^{d}	95.1 ± 0.7 ^{b}	16.3 ± 0.7 ^{c}	37.3 ± 2.9 ^{ab}
'Gisela 5'	7.7 ± 1.0 ^{a}	23.7 ± 1.3 ^{ab}	25.4 ± 1.4 ^{ab}	20.6 ± 0.8 ^{bc}	95.2 ± 0.6 ^{b}	15.0 ± 1.2 ^{e}	35.9 ± 6.2 ^{b}

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

The SSC above 15% was determined for almost all scion-rootstock combinations in 2020, with the exception of fruits harvested on 'Summit'- PF_02_16 trees (Table 6). In 2021, the lowest SSC—about 15% was found in fruits of trees on 'Gisela 5' as well as on PF_02_16. In both years, the SSC was higher for trees grafted on PC rootstock candidates than on PF candidates, with average values of 18.6% and 15.7% in 2021, respectively. Petiole length was within range of 29.5–36.8 mm in 2020, while in the following year varied from 34.3 mm to 39.3 mm. The skin color was determined as red for all scion-rootstock combinations, while mesocarp was described as light red colored. The significant differences were found in the content of acids and sugars, determining the taste of fruits (Table 5). Fruits on both PC rootstock candidates were characterized with medium acidity and medium sweetness levels. Acidity of fruits on PF rootstock candidates was described as low, with low or medium content of sugars. Low acidity and medium sweetness were attributed to fruits on PM candidate, while trees on 'Gisela 5' produced the least tasty fruits with medium acidity and low sweetness levels.

4. Discussion

Temperate fruits cultivation nowadays is a challenging venture which encompasses important aspects such as yield optimization, the achievement of sensory and nutritional quality of fruits, as well as environmentally-friendly usage of resources [33]. Along with the introduction of size-controlling rootstocks for the more efficient cultivation, breeding goals are oriented towards the fruit species and varieties which are adaptable and resistant to various biotic and abiotic stresses, enabling the sustainable fruit production in commercial and individual orchards and gardens, both in rural and urban environment [34]. Sweet cherry development is extremely dependent on climatic factors, and different weather events could affect it at different phenological phases [35,36], such as the poor bud differentiation for the next year during the dry summers or fruit cracking in ripening phase during the wet periods, observed in the conditions of study area [17]. Since precipitation patterns worldwide and in Serbia [37] are strongly affected by climate change and drought events occur more frequently, the water use efficiency is considered an important goal in the sustainable sweet cheery production [12]. In areas of low rainfall where water is a major limiting factor of food production, the production of sweet cherry is not prioritized next to the more calorific crops [38]. In such circumstances, a proper rootstock selection provides the solution—sweet cherry could be grown with minimal inputs and without the consumption of scarce amounts of water, if the rootstock is chosen for its adaptability to changing climate. In these efforts, the research on autochthonous genetic resources of fruit species represents an important objective, since the natural populations are evolving under the pressure of complex conditions prevailing in the natural environment, making them an irreplaceable source of adaptability, resistance and productivity [39].

The observations regarding qualitative traits of trees grafted on different rootstock candidates and 'Gisela 5', varied significantly, which was expected due to the diverse characteristics of investigated rootstock material. Good anchorage strength and absence of suckers are desired features in rootstock selection [17,37]. Although Ogašanović et al. [40] have found weaker anchorage strength and high suckering tendency of plants grafted on 'Oblačinska' sour cherry, our results showed very good anchorage and low or completely absent suckering on PC candidates. Anchorage strength of PF rootstock candidates was also good, accompanied with many to none suckers, opposite to Fogle [41] who pointed out to the poor anchorage and profuse suckering of European ground cherries. The high variability within both cherry populations, previously determined by other studies, opens the possibility to select rootstocks with few or no suckers [42,43]. The excellent anchorage and complete absence of suckers observed for PM candidate and 'Gisela 5' were in accordance with previous findings [44]. Poor vitality of trees on 'Gisela 5' was also found by other authors, as a limiting factor for its utilization as rootstock in areas lacking water availability, due to its lower ability to resume normal growth after being exposed to drought [45,46].

The TCSA measurements are widely used to assess the tree vigor and growth dynamics. Our results showed that the rootstock thickening was the weakest during the experimental period for almost all scion-rootstock combinations, in contrary to the radial growth of scions which had the highest growth rates. The same pattern was observed by

Ljubojević et al. [47], who pointed out to the differences in xylem/phloem areas on rootstock and scion trunk cross-sections, further affecting the transport of water, minerals and assimilates through the plant. The lowest discrepancies between TCSA values of the rootstock and the scion was observed in combination with PF candidates, which could be explained by the ability of *P. fruticosa* to follow the thickening of *Prunus avium* [20,48]. Although the greatest reduction of scion's TCSA in comparison to dwarfing 'Gisela 5' was found in combination with PF rootstock candidates, the dwarfing character could be assigned to all rootstock candidates if only TCSA values were observed. The dwarfing potential of *P. cerasus* and *P. fruticosa* selections in relation to TCSA was also found in previous studies [25,49]. In general, the greatest annual radial growth of all graft parts was found in 2018 compared to 2017 (1.9-fold and 2.4-fold increase of rootstock and scion TCSA, respectively), while the TCSA annual increase during the following years recorded a downward trend. That presumably occurred due to the significantly higher annual precipitation sum in 2018 in comparison to the previous year—the total amount of precipitation was 100 mm higher and distributed evenly throughout the vegetation, while such significant inter-annual differences in the amount of precipitation were not recorded in the following years. In addition to the strong influence of site conditions on plant development [50,51], the decreasing trend of annual radial growth of the trunk was most likely caused by the shift from vegetative to generative phase [52]. The highest increase of tree height from 2017 to 2020 was observed on PF rootstock candidates (1.8-fold, in average), which was in accordance with the highest increase of scion TCSA determined for trees on these candidates (6.2-fold, in average). The lowest increase of both scion TCSA and tree height was observed in combination with 'Gisela 5', which induced the height increase of 40% from third to sixth vegetation. In average, the tree height increment was lower than crown width and depth increase during the four-year period. The highest increase for crown depth values presumably occurred due to more available space between rows than between plants, which enabled plants to grow unhindered in that direction. With regard to the sweet cherries, trees' target dimensions vary depending on training system. For example, the target height for the Tall Spindle Axe training system is 2.5–3 m [53], for Vogel Central Leader 3–3.6 m, while the Spanish Bush training system produces plants with height below 2.5 m [54]. The tree height below 3 m in sixth vegetation make our rootstock candidates suitable both for high density plantings and non-commercial growing in wide range of spaces, facilitating maintenance of grafted sweet cherries and reducing production costs.

In the high density cherry growing, the achievement of an optimum balance between vegetative growth and fruiting is of great importance [55]. In this research, the bud distribution pointed to the noticeable drop in the share of vegetative buds along the two-year-old branches from fourth to sixth vegetation, indicating the need for pruning in order to prevent the displacement of leaf mass to the crown periphery, concurrently improving yield and fruit quality [56]. Our results confirmed the findings of other authors regarding the strong influence of rootstock on the number and distribution of different buds and elements along the one- and two-year-old branches [42,57,58]. In 2020, the highest share of fruit spurs was correlated with the highest achieved yield, while in the next vegetation some trees with high yielding potential failed to obtain high yields, which was presumably related to the marginal position of those trees in the orchard exposed to the strong northeast wind. Despite the high number of fruit spurs per branch recorded on trees on both PC candidates, only candidate PC_02_01/4 induced significantly higher yielding in both years with yield increase in 2021 without jeopardizing fruit mass which was about 8 g in 2020–2021. Among trees on all rootstock candidates and 'Gisela 5', only trees on PC_05_04 achieved lower yield in 2021 compared to the previous year, pointing to the lower rootstock candidate's resistance to the orchard's unfavorable conditions—cold springs and dry summers. Although trees grafted on some other PF rootstock candidates performed very well in the same conditions of water deficit and minimal herbicide usage [25], trees on all three PF candidates studied in this paper failed to achieve yield in the range of that obtained on PC_02_01/4. Though candidate PF_04_09 induced slightly lower yielding than control 'Gisela 5' in 2020, and higher yield in 2021, the fruit mass on this candidate decreased in the sixth vegetation. Pal et al. [55] have found that Mahaleb used as a rootstock in high density sweet cherry plantations delay the entry of trees into bearing period and induce smaller yields. In our trial, both trees on PC and PF candidates performed better than trees on PM candidate, which were characterized with the poor yielding and the lowest fruit mass and dimensions. In general, not all candidates induced the lower fruit mass with the increasing of yield, as found by Blanco et al. [59].

Sweet cherry, characterized by high nutritional value, belongs to fruits mainly consumed in fresh condition. Although the fruit mass is considered to be the main indicator of successful production, the consumers' preferences regarding traits of sweet cherries vary greatly in different regions of the world [60]. Bujdosó et al. [61] have found that the medium fruits with diameters between 21.4 and 25.4 mm, and large fruits with diameters up to 29.8 mm, are preferred by high percentage of customers in some countries, while in other the very large fruit size is sought, indicating that the desired fruit size is a variable production goal. According to Whiting et al. [62], high quality fruits are characterized with fruit width higher than 26 mm. Zheng et al. [63] have found that consumers are willing to pay the greatest premium for sweetness of sweet cherries and the smallest premium for fruit size. Since stone fruit sweetness is usually expressed as soluble solids concentration (SSC) [64], the highest SSC values indicates the sweetest fruits, with recommended minimal values of 15–16% for sweet cherries in general [65,66]. With the raising awareness of consuming public about the impact of their purchases and activities on the environment, the 'sustainable' and 'green' products are becoming more attractive [67]. Thus, the balance between the desirable traits and sustainable agricultural practices should be of major importance in food production. According to other studies [68,69], fruits of 'Summit' cultivar reached mass values of about 8 g on *P. mahaleb* and *P. avium*. The average fruit mass in our trial was about 8 g for two years of fruit assessment. The highest yield in 2021 achieved on PC_02_01/4 (above 4 kg per tree), coupled with the average fruit mass of 7.7 g, exceeded the yield on 'Gisela 5' although the fruit mass was the same on both rootstocks. Although the yield determined in this trial does not guarantee the economic profitability, it indicates the possibility to select more productive rootstock candidates within the investigated autochthonous cherry germplasm. Considering that in the pertinent study pruning was not performed, and the frost protection and irrigation were not implemented in the orchard, the low yielding was not surprise; moreover, the harvested yield provide an insight into the most perspective rootstock candidates which induce yielding even in the unfavorable conditions. Fruit width reached above 26 mm on three rootstock candidates, while the SSC exceeded 15% in all scion-rootstock combinations, which met the above stated standards.

5. Conclusions

The pertinent study showed that in semi-arid climate, with proper rootstock selection, sweet cherry trees could be dwarfing, productive and adaptable without jeopardizing the fruit quality, if production efficiency goals are set to support sustainability. With the raising environmental problems around the globe, the shift from mainly profit-oriented food production to the growing practices which enable environment preservation and resources conservation should be advocated more profoundly. Our results pointed to the high potential of autochthonous *P. cerasus* ecovar. 'Oblačinska' and *P. fruticosa* cherry genotypes, in the context of adaptability to unfavorable conditions. The best performance of sweet cherry trees was determined on rootstock candidate PC_02_01/4, which induced the highest fruit abundance and yielding in both fruiting years, accompanied with satisfactory fruit characteristics and size-control of scions. Our hypothesis that 'Summit' trees could achieve better features on investigated rootstock candidates than when grafted on *P. mahaleb* candidate and 'Gisela 5' in the given environment was partially confirmed—while all candidates induced better performance of grafted cultivar than *P. mahaleb*, when compared to trees on 'Gisela 5' the results varied. Future research should be carried out to investigate the potential of wide spectrum of local autochthonous cherry germplasm, in different regions and in combination with different cultivars to enable the selection of the most perspective genotypes for rootstock breeding purposes.

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