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Protocol for Pre-Selection of Dwarf Garden Rose Varieties

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Abstract: Ornamental plant breeding enables the selection of cultivars with desired features from numerous genotypes; however, this process is time-consuming and resource-demanding. Aiming to establish a pre-selection protocol that can facilitate the selection of dwarf rose varieties, the connection between anatomical and histological characteristics and the vegetative growth of rose cultivars was examined. To assess the adaptive potential of the studied cultivars, intra-annual cambial dynamics were explored relative to the observed meteorological fluctuations during the growing season. The investigation included six garden rose cultivars from the 'Reka' and 'Pixie' collections, bred under semi-arid open-field conditions in Serbia. Plant height ranged from 20 to 68 cm, with differing growth habits and types. Vegetative growth was significantly correlated with the xylem/phloem ratio, the proportion of total vessel area relative to cross-sectional and xylem areas, vessel-related features, and porosity (correlation coefficients up to 0.78). Regeneration via cambial activity and the formation of false rings were observed in five of the six cultivars studied, with meteorological analysis suggesting that precipitation and temperature triggered cambial reactivation. This approach effectively targets key parameters in the selection of dwarf and climate-resilient rose cultivars, facilitating the development of reliable pre-selection criteria.

Keywords: anatomy; cambial activity; climate change; meteorological factors; plasticity; *Rosa* × *hybrida*; selection; vigor; xylem features

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1. Introduction

Miniature roses are a specific group of roses, as their name suggests, smaller in size compared to their full-sized counterparts. Despite their diminutive stature, these roses offer vibrant colors, intricate blooms, and the same alluring fragrance and landscaping features that roses are appreciated for. Besides indoor and landscaping purposes, miniature roses serve in religious offerings and exhibitions [1].

Miniature roses trace their origins back to China, where the first varieties were cultivated. These early miniatures were presumably introduced to Europe in the 18th century, primarily through trade with Asia. Over the years, rose breeders have hybridized and refined these varieties to create the wide range of miniature roses available today. The modern miniature roses are often the result of crossbreeding between dwarf varieties and other rose types, leading to an extensive array of colors, forms, and sizes. Their occurrence is a result of a dominant gene at one major locus [2], and can derive from various rose species and *Rosa* × *hybrida* groups. According to Shepherd [3], dwarf roses originate from *Rosa chinensis* minima while Michonneau et al. [4] state *Rosa semperflorens* 'Minima' as the first of a kind, described in England back in 1815. Regardless the origin and resemblance to all sorts of hybrid tea, floribunda, shrubs, or climbers [5], miniature roses have significant potential for pot growing, cut flowers, and garden and landscape elements, provided that besides ornamental value, they possess disease resistance and winter and drought hardiness.

Due to their compact size, the roses display true dual roles. On one side, miniature roses are ideal for pots, window boxes, and other containers, making them perfect for patios, balconies, and indoor gardening, gaining an added value if proven as edible. On the other hand, compact growth habit makes them an excellent garden border element, adding color and structure to the edges of garden beds. In addition, miniature roses can be a central piece of rock gardens, 'alpinetums', where their small size and vibrant blooms create a striking contrast with rocks and other hardy plants.

Breeding ornamental plants is a long and uncertain process, and thus some kind of oriented selection, be it marker, biochemically or anatomically assisted, is necessary. Given the current challenges, including climate change and the need for resource conservation, it is highly advisable to direct selection of ornamental species that are not only adapted to elevated temperatures but also require minimal inputs for production [6]. Garden roses, like many other high-value horticultural plants, are typically cultivated in one climatic region but marketed across diverse climates [7]. The Rosa genus, as a whole, exhibits a broad genetic foundation for tolerance to abiotic stress, which is evident in its wide ecological distribution. Roses demonstrate resilience or tolerance to various environmental stresses, including salinity, drought, and low humidity, combined with high temperatures, and intense light [8]. However, water shortage can notably limit rose growth and development, impairing the landscaping potential. In the face of emerging global challenges, the delicate equilibrium between water availability and demand necessitates thoughtful strategies for mitigation and careful water management. One of the nature-based solutions mitigating the water scarcity is the selection and usage of ornamentals with pronounced drought tolerance or even resistance. However, evaluating the ground cover roses in the semi-arid conditions in Italy, Amoroso et al. [9] noted that there are discrepancies due to the differences in the initial selection and final application environments. Namely, both Amoroso et al. [9] and Michonneau et al. [4] emphasize that most of the miniature roses are bread in Northern Europe, characterized by higher humidity and better-distributed precipitation.

Due to the limitations associated with dwarf roses' cultivation in drought conditions, this study aimed to assess the anatomical properties of six cultivars bred in Serbian semi-arid open-field conditions. Upon the anatomical characterization, a pre-selection protocol, facilitating rapid selection of the most promising genotypes among tens of thousands of seedlings each year, for adverse environmental conditions shall be defined. The final goal is a cost-effective, easily repeatable, fast screening of seedlings both in field and greenhouse conditions.

2. Materials and Methods

2.1. Plant Material and Site Conditions

The plant material included six tetraploid (4n) garden rose cultivars (*Rosa* × *hybrida* L.), bred by the private breeding company 'Pheno Geno Roses' and grown in the company's open experimental field located in Temerin, Northern Serbia (45°24′19″ N 19°53′13″ E). One cultivar, named 'Morava Reka,' belongs to the 'Reka' collection, while the other five cultivars are part of the 'Pixie' collection, including 'Blush Pixie', 'Coral Pixie', 'Gaudi Pixie', 'Mauve Pixie', and 'Milky Pixie' (Figure 1). The cultivar 'Morava Reka' is derived from a 'Raspberry Royal', as the seed parent was open-pollinated. The parentage of cultivars from the 'Pixie' collection is shown in Table 1.

Table 1. The genetic background of cultivars from the 'Pixie' collection.

Cultivar Name	Seed Parent	Pollen Source
'Blush Pixie'	'Raspberry Royal'	'Pink Tiara'
'Coral Pixie'	'Austriana'	'Raspberry Royal'
'Gaudi Pixie'	'Winnipeg Parks'	'David Thompson'
'Mauve Pixie'	'Violette Parfumee'	'Raspberry Royal'
'Milky Pixie'	'Diamond Border'	'Amber Cover'

Figure 1. Garden rose cultivars from the 'Morava Reka' and 'Pixie' collections: (**A**) 'Morava Reka'; (**B**) 'Blush Pixie'; (**C**) 'Coral Pixie'; (**D**) 'Gaudi Pixie'; (**E**) 'Mauve Pixie'; (**F**) 'Milky Pixie'.

The trial was conducted in an area with a typical continental climate, characterized by extremely warm summers and cold winters. It was established in the autumn of 2021, by on-site bud grafting onto *Rosa laxa*. The grafted plants were planted with a 10 cm distance between plants in the row and 1 m distance between rows. Plants were not irrigated nor chemically treated. Morphological characterization was conducted on 10 plants per cultivar in the late spring and early summer of 2023, while sampling for the anatomical and histological investigation was performed on five plants per cultivar later on in December 2023, during winter dormancy. Three biological replicates—three branches from approximately 120° angles around the bush—were taken from each of the five plants. Meteorological data were collected by a weather station installed in the field, Metos AG/CP/DD (Pessl Instruments, Weiz, Austria), located within a 10 km radius of the experimental field. In the year of sampling, the average annual air temperature was 13.46 °C, with maximum daily temperatures reaching up to 37.96 °C and winter temperatures dropping to −10.25 °C (Table 2). The annual precipitation total was above 700 mm, with the highest amount of rain recorded in May (95.6 mm).

Table 2. Main meteorological parameters of study area during 2023.

	Air Temperature (°C)			Relative Humidity (%)	Precipitation Sum (mm)
	Avg	**Max**	**Min**		
January	4.61	18.45	−1.64	15.16	77.6
February	3.61	18.59	−10.25	0.04	67.6
March	8.87	25.25	−3.02	29.64	27.6
April	10.36	23.46	−1.42	73.34	66.2
May	16.78	28.84	3.69	76.38	95.6
June	20.72	36.45	10.21	77.04	41.4
July	24.14	37.06	10.26	69.55	50.2
August	23.11	37.96	9.41	73.97	45.6
September	20.59	33.99	9.99	70.39	84.2
October	16.07	28.9	−1.19	70.95	88.4
November	7.87	21.76	−3.56	85.83	58.8
December	4.75	20.91	−7.76	88.74	48.4

2.2. Morphological, Anatomical, and Histological Assessment

The morphological characterization included both descriptive and metric plant and flower parameters, as previously described by Simin at al. [10]. It was conducted in June upon the completion of vegetative growth, according to the UPOV protocol for roses (*Rosa* L.) [11]. Descriptive plant features encompassed growth type and growth habit. Additionally, growth height (cm) was measured. Growth type was classified into one of the following categories: miniature, dwarf, bed, shrub, climber, and ground cover. Growth habit was assessed based on five different types: upright, semi-upright, intermediate, moderately spreading, and strongly spreading. Flower characterization included the evaluation of the following features: flower type, color group, color of center, shape, profile of upper and lower parts, density of petals, and fragrance. The diameter (cm) of each flower and the number of petals were recorded. For each cultivar, five flowers from each of ten replicate plants were assessed. The flowering period was also monitored.

Anatomical and histological characterization was performed on one-year-old stems, which were preserved in 60% ethanol (Reahem Ltd., Novi Sad, Serbia) with the addition of 10% glycerin (Centrohem Ltd., Stara Pazova, Serbia) until sectioning [12]. The stems were cross-sectioned around the fourth internode. Microscopic observations were conducted using a Motic Digital BA310 biological light microscope (Motic China Group Co., Ltd., Xiamen, China) equipped with a built-in digital camera, allowing images to be saved at 40×, 100×, and 400× magnifications. The accompanying software, Motic Images Plus 2.0, was used for measurements.

Anatomical features were measured on four radial segments of each cross-section, positioned 90° apart. The following features were measured: stem diameter (mm), cross-section area (CSA, mm^2), pith area (mm^2), xylem area (mm^2), vascular cambium area (mm^2), phloem area (mm^2), and total area of sclerenchyma, parenchyma, collenchyma, cork cambium, and epidermis (mm^2). Non-vascular tissues, as described by Morales-Orellana et al. [13], were not separately observed due to their irrelevance to the subject of the study. The proportion of both xylem and phloem areas was calculated relative to the CSA (%). The xylem/phloem ratio was determined by dividing the total xylem area by the total phloem area. Depending on the xylem width, histological parameters were measured in 1 to 3 visual fields on each radial segment. Vessel area, ray area, and the area without vessels and ray cells were measured, and their proportions were then determined relative to both CSA and xylem area (%). The average vessel lumen area (VLA, µm^2) and vessel frequency (VF), defined as the number of vessels per mm^2, were calculated, along with xylem porosity (%). Vessel distribution was determined for each cultivar by classifying the vessels into three size classes: size-class I—vessels with a VLA less than 300 µm^2, size-class II—vessels with a VLA between 300 and 700 µm^2, and size-class III—vessels larger than 700 µm^2. The number of vessels belonging to each size class was presented as the percentage of the total number of vessels. Due to the observation of three distinct xylem zones (rings) in 'Morava Reka' stems, VLA, VF, and vessel distribution were determined for the inner, middle, and outer xylem zones.

2.3. Statistical Analyses

Statistical analyses of the data were performed using Statistica 14 software (Tibco, Palo Alto, CA, USA). Morphological, anatomical, and histological data were analyzed by one-way Analysis of Variance (ANOVA) and Tukey's Honest Significant Difference test, with a significance level of $p \leq 0.05$. Before performing ANOVA, the normality of the distribution was tested and confirmed using the Shapiro–Wilk test, which supported the results of the previously conducted visual inspection of the Q-Q plots. Additionally, correlation analysis was performed to explore the relationships between the investigated parameters, with the strength of interdependence among components indicated by Pearson's correlation coefficient ($p < 0.05$).

3. Results

3.1. Morphology and Anatomy of the Investigated Rose Cultivars

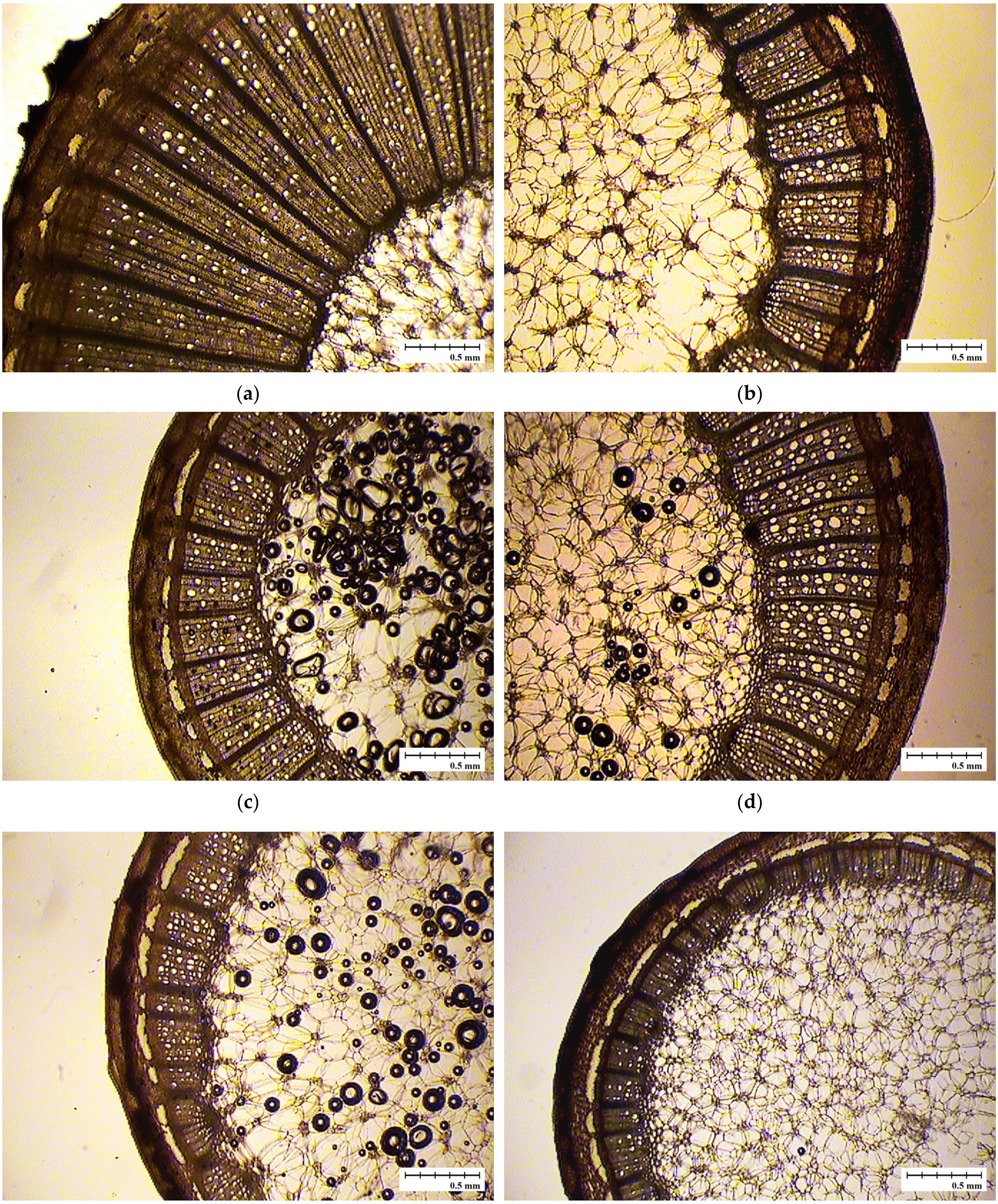
Morphological investigation of the studied rose cultivars showed a variety of growth types and habits (Table 3). Four cultivars were characterized as a shrub, while the remaining two were described as a ground cover. Growth habit varied from semi-upright to upright, while ground covers were moderately or strongly spreading. The highest height was measured in ‘Gaudi Pixie’ and ‘Coral Pixie’ (above 60 cm), while ‘Milky Pixie’ was characterized by the lowest height (20 cm). All cultivars featured double flowers with a variety of colors in both the petals and the center of the flower. The observed flower shapes were star-shaped, rounded, and irregularly rounded. The diameter ranged from 2.5 cm in ‘Blush Pixie’ to 6.5 cm in ‘Gaudi Pixie’, while the number of petals varied from 22.2 to 66.4 on average. Five out of six cultivars had absent or weak fragrance, with only ‘Gaudi Pixie’ exhibiting a medium fragrance. The full flowering period was similar for all investigated cultivars, lasting from the first week of June to mid-September.

Table 3. Morphological parameters of investigated rose cultivars.

	‘Morava Reka’	**‘Blush Pixie‘**	**‘Coral Pixie’**	**‘Gaudi Pixie’**	**‘Mauve Pixie’**	**‘Milky Pixie’**
Plant characteristics						
Growth type *	shrub	ground cover	ground cover	shrub	shrub	shrub
Growth habit	upright	strongly spreading	moderately spreading	intermediate	semi upright	intermediate
Height (cm)	40 c	52 b	67 a	68 a	50 b	20 d
Flower characteristics						
Type	double	double	double	double	double	double
Color group	46A	068B	045A	N066B	061B	NN159
Color of center	red	pink	red	pink	purple	white
Shape	star-shaped	rounded	irregularly rounded	rounded	rounded	star-shaped
Upper part profile	flat	flat	flat	flattened convex	flat	flattened convex
Lower part profile	concave	flat	flat	flat	flat	flat
Diameter (cm)	4.9 b	2.5 c	3.9 b	6.5 a	4.4 b	3.8 b
Number of petals	22.2 c	44.2 b	41.6 b	64.4 a	66.4 a	60 a
Density of petals	medium	dense	loose	medium	loose	medium
Fragrance	absent or weak	absent or weak	absent or weak	medium	absent or weak	absent or weak

* Measurements were performed during June 2023. Qualitative assessment was conducted according to the UPOV protocol for roses [13]. Different letters designating mean values within a row indicate significant differences between cultivars, according to Tukey’s Honest Significant Difference (HSD) test ($p \leq 0.05$). Tukey’s HSD test derived from one-way ANOVA as a post hoc analysis.

The investigation of stem cross-sections showed significant differences in anatomy of the studied cultivars (Figure 2, Table 4). The highest stem diameter was recorded in ‘Morava Reka’ (6.70 mm), while the lowest value was measured in ‘Milky Pixie’ (4.45 mm). Cross-section area (CSA) varied from 15.68 mm^2 to 35.62 mm^2. The share of different tissues within the total CSA differed considerably between cultivars. The highest xylem areas were observed in ‘Morava Reka’ stems, occupying 48.52% of CSA and in ‘Gaudi Pixie’ stems, where xylem area occupied 37.91% of CSA. The lowest share of xylem in relation to total CSA was measured in ‘Milky Pixie’ stems, reaching only 16% on average. The phloem area occupied from 3.95% of CSA in ‘Milky Pixie’ stems to 10.31% of CSA in the stems of ‘Morava Reka’. The xylem/phloem ratio ranged from 3.76 to 6.32.



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

(**c**) (**d**)

(**e**) (**f**)

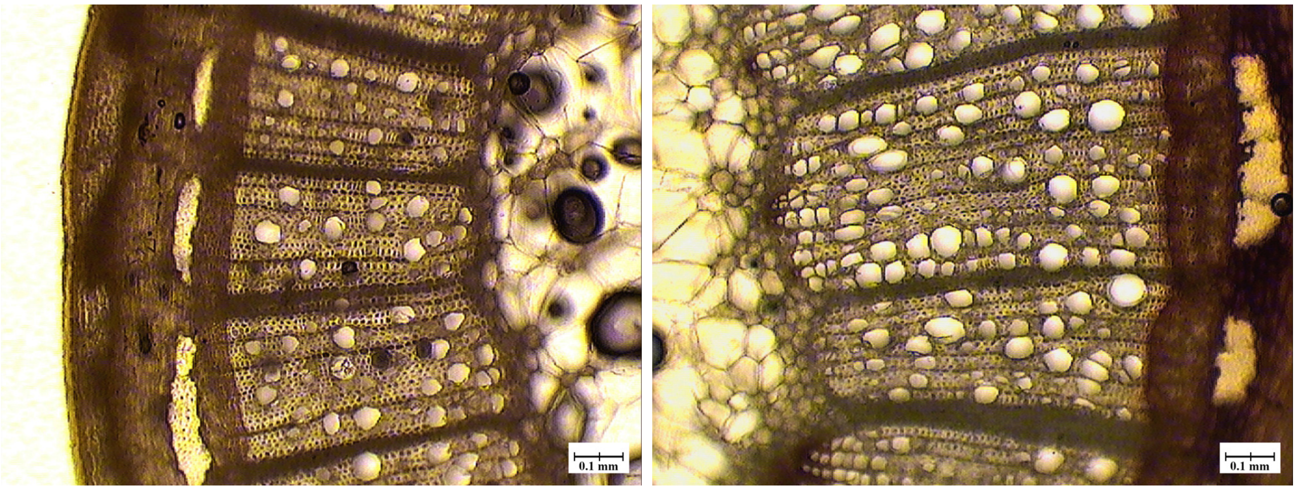
Figure 2. One-year-old stem cross-sections of investigated cultivars at 40× magnification: (**a**) 'Morava Reka'; (**b**) 'Blush Pixie'; (**c**) 'Coral Pixie'; (**d**) 'Gaudi Pixie'; (**e**) 'Mauve Pixie'; (**f**) 'Milky Pixie'.

Table 4. Stem anatomical parameters of ‘Morava Reka’ and cultivars from ‘Pixie’ collection.

	‘Morava Reka’	‘Blush Pixie‘	‘Coral Pixie’	‘Gaudi Pixie’	‘Mauve Pixie’	‘Milky Pixie’
Stem diameter (mm)	6.70 ± 0.77 ** a	5.45 ± 0.59 ab	5.61 ± 0.67 ab	5.75 ± 0.27 ab	5.09 ± 0.06 b	4.45 ± 0.41 b
Cross-section area (CSA, mm^2)	35.62 ± 8.18 a	23.53 ± 5.12 ab	25.03 ± 5.93 ab	26.06 ± 2.46 ab	20.30 ± 0.52 b	15.68 ± 2.92 b
Pith area (mm^2)	6.88 ± 0.87 a	10.24 ± 2.99 a	10.66 ± 2.78 a	8.01 ± 1.52 a	10.01 ± 1.58 a	8.17 ± 1.57 a
Xylem area (mm^2)	17.70 ± 6.48 a	5.94 ± 1.30 b	7.01 ± 2.66 b	9.89 ± 0.55 ab	4.68 ± 1.45 b	2.55 ± 0.77 b
Vascular cambium area (mm^2)	0.59 ± 0.16 a	0.30 ± 0.05 b	0.30 ± 0.10 b	0.29 ± 0.02 b	0.23 ± 0.02 b	0.21 ± 0.03 b
Phloem area (mm^2)	3.64 ± 0.70 a	1.67 ± 0.45 bc	1.11 ± 0.32 bc	1.84 ± 0.27 b	0.95 ± 0.14 bc	0.64 ± 0.24 c
Other tissues (mm^2) *	6.80 ± 1.01 a	5.39 ± 0.61 ab	5.94 ± 1.51 ab	6.03 ± 0.50 ab	4.44 ± 0.37 b	4.11 ± 0.35 b
% of xylem on CSA	48.52 ± 6.85 a	25.25 ± 2.32 bc	28.00 ± 8.53 bc	37.91 ± 3.22 ab	22.93 ± 6.59 bc	16.00 ± 1.77 c
% of phloem on CSA	10.31 ± 1.30 a	7.06 ± 1.13 b	4.43 ± 0.42 c	7.03 ± 0.38 b	4.69 ± 0.63 bc	3.95 ± 0.76 c
Xylem/phloem ratio	4.82 ± 1.02 ab	3.76 ± 0.28 b	6.32 ± 1.52 a	5.42 ± 0.72 ab	4.88 ± 0.86 ab	4.17 ± 0.40 ab

* Tissues include sclerenchyma, parenchyma, collenchyma, cork cambium, and epidermis. ** Mean values are followed by ±standard deviation value. Different letters designating mean values within a row indicate significant differences between cultivars according to Tukey’s Honest Significant Difference (HSD) test ($p \leq 0.05$). Tukey’s HSD test derived from one-way ANOVA as a post hoc analysis.

The characteristics of xylem tissue displayed notable differences among the cultivars (Table 5). The total area under the vessels in relation to stem CSA varied from 1.86% in ‘Milky Pixie’ stems to 10.32% in ‘Gaudi Pixie’ stems. Rays occupied less than 10% of CSA in all cultivars. In relation to xylem area, the vessel area counted from 11.47% in ‘Milky Pixie’ stems to 27.03% in ‘Gaudi Pixie’ stems (Figure 3). The area occupied by rays accounted for approximately 21% of the total xylem area on average. The highest proportion of area without vessels and ray cells relative to the xylem area was observed in ‘Milky Pixie’, ‘Morava Reka’, and ‘Coral Pixie’ (above 60%). The average vessel lumen area (VLA) ranged from 386.14 to 818.02 μm^2, while the vessel frequency (VF) varied between 200.43 and 393.61. There was no significant correlation between the VLA and VF, meaning that an increase in VLA does not necessarily correspond to a decrease in VF, and vice versa. The lowest xylem porosity was calculated for ‘Milky Pixie’ (11.47%), significantly different from other three cultivars, according to Tukey’s test. The highest porosity was found in ‘Gaudi Pixie’ and ‘Blush Pixie’ stems, reaching 27.03 and 21.51% on average, respectively.



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

Figure 3. Differences in total vessel area within stems of (**a**) ‘Coral Pixie’ and (**b**) ‘Gaudi Pixie’, with the notable two false rings in one growing season. The sections were observed at 100× magnification.

Table 5. Xylem traits of ‘Morava Reka’ and cultivars from ‘Pixie’ collection.

	‘Morava Reka’	**‘Blush Pixie‘**	**‘Coral Pixie’**	**‘Gaudi Pixie’**	**‘Mauve Pixie’**	**‘Milky Pixie’**
Vessel area on CSA (%)	8.01 ± 1.59 * ab	5.40 ± 0.63 bc	4.96 ± 1.89 bcd	10.32 ± 1.28 a	3.87 ± 0.50 cd	1.86 ± 0.40 d
Ray area on CSA (%)	9.40 ± 1.10 a	5.48 ± 1.08 bc	5.08 ± 0.77 bc	7.69 ± 0.80 ab	6.12 ± 2.34 abc	3.42 ± 1.35 c
A(-)VR on CSA (%)	31.24 ± 4.27 a	14.39 ± 2.07 b	18.06 ± 5.66 b	20.09 ± 1.70 b	12.98 ± 4.43 b	10.79 ± 0.11 b
Vessel area relative to Xy (%)	16.38 ± 1.27 bc	21.51 ± 3.53 ab	17.27 ± 1.90 bc	27.03 ± 1.62 a	17.42 ± 3.16 bc	11.47 ± 1.25 c
Ray area relative to Xy (%)	19.37 ± 1.18 a	21.61 ± 3.06 a	18.74 ± 3.32 a	20.26 ± 2.30 a	26.31 ± 6.26 a	20.88 ± 5.82 a
A(-)VR relative to Xy (%)	64.24 ± 0.75 ab	56.88 ± 5.58 abc	63.99 ± 1.42 ab	52.71 ± 0.69 c	56.27 ± 5.20 bc	67.64 ± 6.98 a
Vessel lumen area (μm^2)	818.02 ± 65.65 a	760.37 ± 70.37 ab	595.63 ± 142.67 bc	688.93 ± 48.32 ab	561.68 ± 70.69 bc	386.14 ± 77.65 c
Vessel frequency	200.43 ± 8.23 c	282.11 ± 26.83 bc	297.35 ± 46.84 abc	393.61 ± 35.96 a	314.44 ± 71.51 ab	301.47 ± 32.94 abc
Xylem porosity (%)	16.38 ± 1.27 bc	21.51 ± 3.53 ab	17.27 ± 1.90 b	27.03 ± 1.62 a	17.42 ± 3.16 b	11.47 ± 1.25 c

CSA, cross-section area; A(-)VR, area without vessels and ray cells; Xy, xylem area. * Mean values are followed by ±standard deviation value. Different letters designating mean values within a row indicate significant differences between cultivars, according to Tukey’s Honest Significant Difference (HSD) test ($p \leq 0.05$). Tukey’s HSD test derived from one-way ANOVA as a post hoc analysis.

Calculated VLAs and VFs reflected the distinctive patterns of vessel distribution within investigated cultivars (Figures 4 and 5). ‘Morava Reka’ showed gradual increase in the vessel size, with the lowest number of vessels belonging to size-class I and the highest number of vessels larger than 700 μm^2. On the other hand, ‘Milky Pixie’, as the cultivar with the lowest average VLA, recorded a decrease in the number of vessels as their dimensions increased. A similar pattern was observed in ‘Coral Pixie’ but with smaller differences in the presence of vessels belonging to different classes. In both ‘Blush Pixie’ and ‘Gaudy Pixie’, vessels with dimensions within the range of 300–700 μm^2 were the least present compared to the other two classes (less than 25%), while in the stems of ‘Mauve Pixie’, vessels belonging to size-class II were the most prevalent, accounting for 37.60% of the total number. In general, the highest proportion of vessels smaller than 300 μm^2 was observed in ‘Milky Pixie’ and ‘Gaudy Pixie’ (51.98 and 41.04%, respectively), while vessels larger than 700 μm^2 were most numerous in ‘Morava Reka’ and ‘Blush Pixie’ (45.44 and 44.70%, respectively).

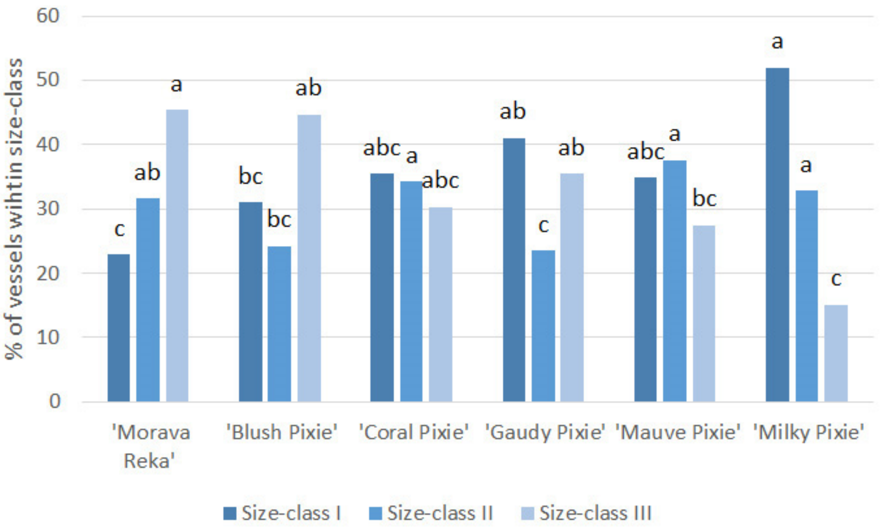
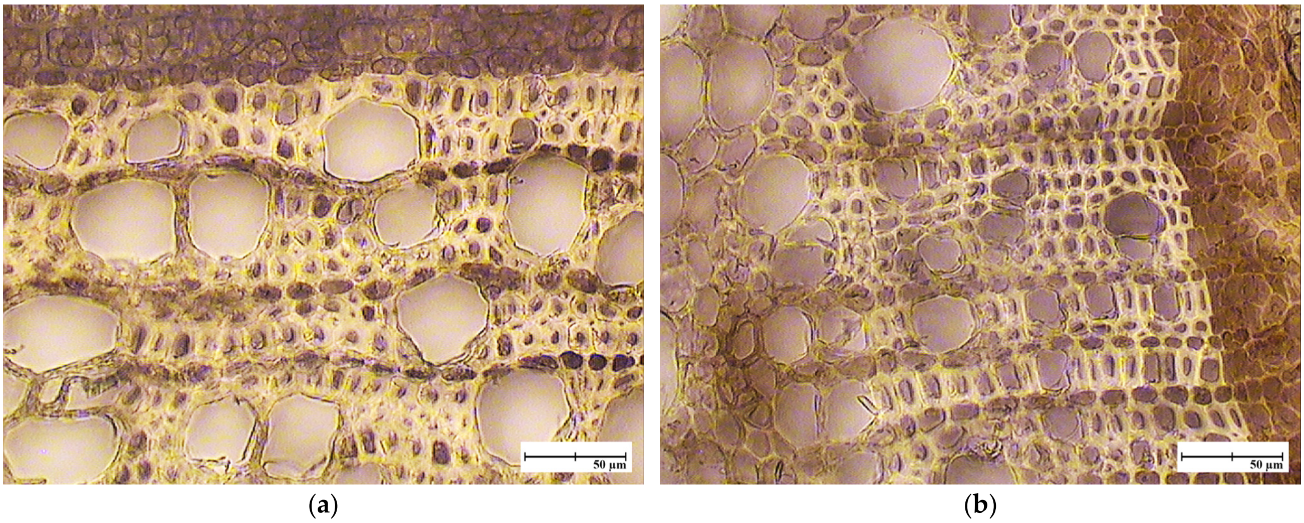


Figure 4. Distribution of vessels within size-classes according to calculated vessel lumen areas. Different letters designating mean values within one size-class indicate significant differences between cultivars, according to Tukey’s Honest Significant Difference (HSD) test ($p \leq 0.05$). Tukey’s HSD test derived from one-way ANOVA as a post hoc analysis.



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

Figure 5. (**a**) The notable presence of large vessels (highest number of vessels with area above 700 µm^2) within 'Blush Pixie' stems and (**b**) 'Milky Pixie' as a cultivar with the highest number of vessels with area below 300 µm^2. The sections were observed at 400× magnification.

The correlation analysis conducted between the assessed anatomical and histological features and plant height, which is one of the main characteristics reflecting plant vigor, identified key parameters important for the pre-selection process in the development of dwarf cultivars. Although all studied cultivars were characterized as low-vigorous, variations in growth types and spreading intensity provided sufficient material for analysis. The cross-sectional and xylem features most significantly correlated with plant height were as follows: xylem/phloem ratio (r = 0.66), proportion of total vessel area relative to CSA (r = 0.60), proportion of total vessel area relative to xylem area (r = 0.78), VLA (r = 0.44), VF (r = 0.46), xylem porosity (r = 0.78), and the proportion of vessels larger than 700 µm^2 within the total number of vessels (r = 0.42) (see Supplement Table S1).

3.2. Cambial Plasticity and the Formation of Growth Rings

The pattern of xylem elements observed in one-year-old stems showed the differences in cambial activity between cultivars throughout the vegetation period (Figure 2). The most dynamic activity was observed in 'Morava Reka' stems, where three xylem rings emerged as a result of stops and starts in the vessel production (Figure 2a). Although not so precisely distinguished, the rings can also be noticed in the other cultivars, with the exception of 'Milky Pixie', characterized by narrow xylem area. The reactivated production of vessels is associated with the emergence of larger vessels, whose size gradually decreases, mimicking the shift of early and latewood conductive element succession in semi-ring porous species.

The vessel traits within the three distinct xylem zones (rings) observed in the stems of 'Morava Reka' expressed no statistically significant differences (Table 6). The VLA varied from 792.77 µm^2 in the inner zone to 879.48 µm^2 in the outer zone, while the VF was positively correlated with VLA, reaching up to 221.11 in the oldest xylem zone. With regard to vessel distribution, all zones were characterized by the lowest proportion of vessels under 300 µm^2 (18.60–24.95%) and the highest proportion of the largest vessels (42.36–49.85%).

The analysis of meteorological parameters indicated a possible connection with precipitation distribution during the growing season and cambial reactivation. Figures 6 and 7 show precipitation fluctuations from the beginning of March to the end of September. In the first months of the growing season (March and April), precipitation occurred in a continuous pattern, with the longest dry period lasting 10 days and a total precipitation sum of 93.8 mm. During this period of vegetative growth, an initial xylem was produced. The following two months, May and June, were characterized by a total of 137 mm of precipitation, including one rainy episode from 11 to 18 May that brought 48.8 mm of rain. Although temperatures increased as summer progressed and total precipitation in June was only 41.4 mm, the period without rain did not exceed 6 days. With extreme temperatures above 37 °C, July and August brought dry weather and a small amount of rain, causing the first cambial activity stop around 1–15 July and 26 August to 16 September. The third decade of September was characterized by 79.2 mm of precipitation, which, combined with warm weather, presumably reactivated the cambium, leading to the formation of new vessels, marked as the third ring.

Table 6. Vessel features within three xylem zones (rings) on the stem cross-section of 'Morava Reka' cultivar.

	Vessel Lumen Area (μm^2)	Vessel Frequency	% of Vessels < 300 μm^2	% of Vessels from 300 to 700 μm^2	% of Vessels > 700 μm^2
Inner xylem zone	792.77 ± 142.43 * a	221.11 ± 17.51 a	24.95 ± 6.47 a	25.48 ± 6.27 a	49.58 ± 9.76 a
Middle xylem zone	820.72 ± 96.21 a	201.85 ± 25.56 a	18.60 ± 4.85 a	36.58 ± 8.87 a	44.82 ± 7.64 a
Outer xylem zone	879.48 ± 23.26 a	178.31 ± 22.40 a	24.61 ± 5.83 a	33.03 ± 7.18 a	42.36 ± 3.74 a

* Mean values are followed by ±standard deviation value. Different letters designating mean values within a column indicate significant differences between zones, according to Tukey's Honest Significant Difference (HSD) test ($p \leq 0.05$). Tukey's HSD test derived from one-way ANOVA as a post hoc analysis.

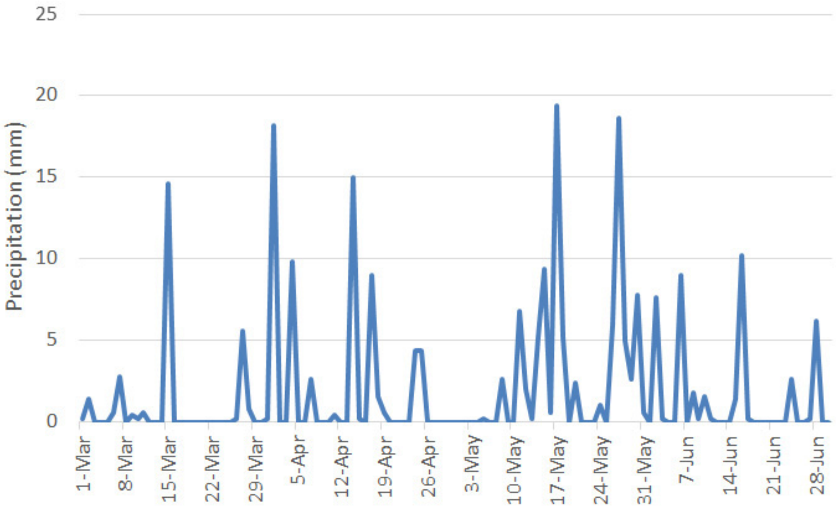


Figure 6. Daily precipitation sums during the period March–June of 2023.

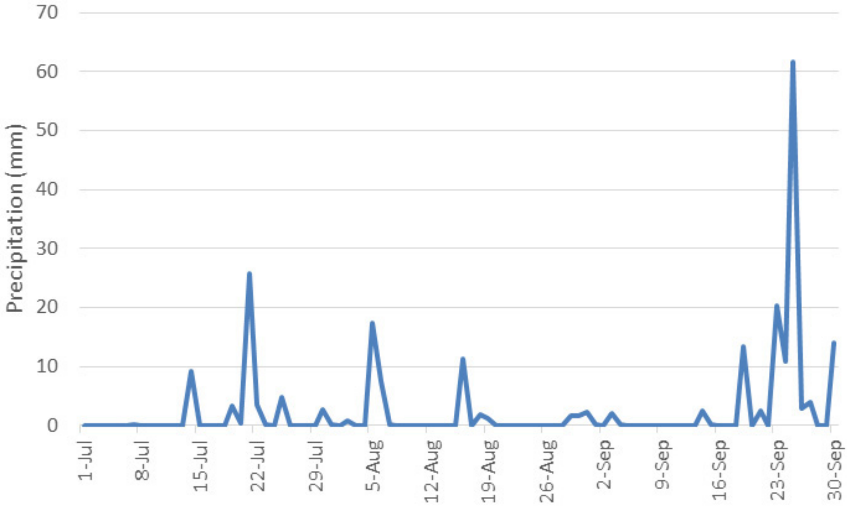


Figure 7. Daily precipitation sums during the period July–September of 2023.

4. Discussion

Perennial plants' breeding and selection are time-, space-, and resource-consuming activities with unpredictable outcomes, especially if only one model plant and one strategy are adopted during the outcomes' definitions. Among them, garden roses encompass a wide spectrum of cultivars bred for different purposes. As traits directly linked to rose cultivation potential, plant vigor and habit represent the first step in selecting desirable cultivars. Investigation of root and stem anatomy at the cross-sectional level, as well as individual xylem traits, could guide breeders' decisions when selecting dwarf cultivars [14]. Some studies have investigated the anatomical features of differing rose cultivars for various purposes [15,16]; however, to the authors' knowledge, there are no studies that link anatomy to plant height and growth habit of roses.

Pertinent study showed that the plant height of the investigated rose cultivars correlates mainly with histological xylem features. At the cross-sectional level, only the xylem/phloem ratio significantly correlated with height, which is consistent with previous studies [17,18]. The area that vessels occupy relative to both total CSA and secondary xylem area was highly indicative of cultivar height. Previously, this parameter was found to be useful in determining plant vigor [19]. Vessel-related traits, such as VLA and VF, as well as xylem porosity, which depend on these traits, also showed a strong correlation with morphological characterization. However, porosity was not evaluated as a reliable parameter when assessing plant vigor, according to Zorić et al. [19]. Due to freezing and drought-induced embolism, the calculated conductive area per unit of stem cross-section tends to be non-functional and is subject to change. Miniature roses, due to their low overall bush height are prone to both summer and winter stressful influences. For this reason, cambial activity and capability of fast responses by novel conductive element production immediately after the stressor's ending is of great importance when setting the breeding objectives.

The amount of water conducted through the plant indisputably depends on the plant's overall physical appearance, including height, growth habit, and level of spreading [20]. Among the studied cultivars, 'Gaudi Pixie' had the highest vigor, supported by the highest values of the proportion of vessel area relative to CSA and xylem area, VLA and xylem porosity, and even VF. The vigor–anatomy connection was also reliably observed in the case of 'Milky Pixie', a shrub characterized by the lowest height, which had significantly lower values for the parameters mentioned. However, despite its lower height compared to other cultivars (with the exception of 'Milky Pixie'), 'Morava Reka' achieved an average VLA of 818.02 μm^2 and a share of vessel area comparable to that of 'Gaudi Pixie'. Furthermore, large vessels (>700 μm^2; 45%), coupled with a VF of 282.11, were associated with the strongly spreading ground cover 'Blush Pixie'. In general, larger vessels are associated with fast water supply to lower heights and numerous branching points, which might have ensured uninterrupted water flow through the plant, despite the increased risk of cavitation under water stress [21].

Interestingly, the highest percentage of the largest vessels (>700 μm^2; 45%), was determined in varieties 'Morava Reka' and 'Blush Pixie', both resulting from the crossing where 'Raspberry Royal' served as the mother plant. When the same variety is used as a pollen donor, this consistency could not be observed, which is a valuable information for future breeding choices. Regarding the breeding activities, pertinent study determined that if only dwarfness is to be considered as the final goal, porosity would be the main feature during the anatomically assisted selection. In the first stage, field conditions, easily assessable features like CSA and mere secondary xylem area can be applied, while laboratory investigations (second stage) of VLA and porosity can supplement and fortify those findings. In that manner significant number of genotypes can be discarded and a core collection of promising genotypes can be further investigated, providing valuable material and human resource savings. However, if adaptive miniature garden roses are being sought, anatomy must be complemented with cambial dynamics observations during the drought and rainy episodes.

Regeneration through cambial activity and false ring formation was noted in five out of the six investigated cultivars, making them suitable for landscaping purposes, while 'Milky Pixie' could be recommended for more controlled cultivation in private gardens and greenhouse cultivation. In the study of Williams et al. [22], under water shortage, miniature roses could restore the stomatal conductance and photosynthesis rates similar to control, non-stressed plants. In the subsequent study [23], it was additionally proven that two miniature rose cultivars employed different drought-tolerance mechanisms—osmotic adjustment ('Apollo' variety) or modified stomatal closure ('Charming' variety). Similarly, in our study, miniature roses were grown in open-field conditions without irrigation but did not express permanent withering symptoms, which we can associate with anatomical level of adaptiveness. The reactivation of cambial activity was most evident in the stems of 'Morava Reka', which formed three distinct growth rings within a single growing season. This allowed for further analysis of xylem features across these rings. The absence of significant differences in vessel-related traits between the rings presumably indicates this cultivar's ability to reactivate the cambium when environmental conditions become favorable, demonstrating strong regenerative potential after drought periods followed by extremely high temperatures, regardless of when such conditions arise. A study conducted by Vaganov et al. [24] showed that xylem differentiation is linked to climatic factors, including temperature, moisture, and solar radiation. The analysis of tree-ring anatomy and seasonal meteorological changes indicated that conduit dimensions in growth rings depend on fluctuations throughout the growing season, where the limiting or activating factor for cambial activity is not always the same. In the case of 'Morava Reka', it can be assumed that a similar amount of precipitation combined with favorable temperatures activated the cambium at three different times. After the optimum temperature was surpassed, vessel diameter likely began to decrease until vessel production fully ceased. This assumption aligns with a previous study by Vaganov et al. [24], which found, for example, that the tracheid dimensions of earlywood in *Pinus densiflora* increase with temperatures from 6–8 °C to 16–18 °C, after which they start to decrease. The two rings presumably formed in spring and early summer, before the onset of high air temperatures, and were characterized by greater width than the third growth ring, which was likely formed in early autumn. It is assumed that lower temperatures at the beginning of October halted xylem production, resulting in the reduced ring width. Türker et al. [25] found that cambial activity in *Rosa canina* in Turkey strongly correlated with environmental factors, including temperature and precipitation. They observed that xylem formation began in the third week of April, forming spring wood, while summer wood production started toward the end of May and early June, and ceased by the end of July. Although there is no obvious pattern in temperature influence on the formation of growth rings in 'Morava Reka', many studies have confirmed the importance of temperature for xylem production [26,27]. Gričar et al. [28] highlighted site-specific growth sensitivities to environmental stimuli, showing that various conditions can affect the radial growth of the same species, with more intense responses to climatic variations in more extreme conditions [29]. Our assumptions are also supported by Ren et al. [30], who suggest that precipitation during late spring and summer can trigger xylogenesis, while extremely dry conditions may delay it, reflecting an adaptive strategy to avoid hydraulic failure. This aligns with a previous study conducted by Ljubojević and Narandžić [31], which demonstrated that root cambial activity reactivation in cherry was initiated by an intense precipitation event. This event occurred after a 39-day dry summer period and resulted in the formation of a new growth ring characterized by large vessels, comparable in size to those typically formed in early spring. Dinella et al. [32] and Wang et al. [33] recognize the interaction between precipitation and temperature as a major driver of xylem formation, emphasizing the complex factors influencing cambial activity. Comparing the rose vascular cambium changes upon the chemical, mechanical, and water stresses, Michonneau et al. [4] noted that much larger vessels under water limitations were generated. In this study the number and the diameter of xylem vessels notably increased upon the water stress, implying their cambial plasticity, as proven in our study.

5. Conclusions

Dwarf garden roses fit almost any private and public green area, be it indoor or outdoor gardening, due to compact growth and aesthetic value. However, dwarf roses derive from various crosses, resulting in tens of thousands of hybrids in one cycle, making the selection process time- and resource-consuming. Additionally, their compact growth makes them prone to drought or freezing/thawing induced embolism and water supply failure. Due to the listed advantages and limitations, this study aimed to assess the anatomical properties of six cultivars bred in Serbian semi-arid open-field conditions. It was noted that for the extremes—the most vigorous 'Gaudi Pixie' and the most minature 'Milky Pixie'—anatomical features (proportion of vessel area relative to CSA and xylem area, VLA and xylem porosity) reliably reflect final shrub size. Besides this simple anatomy-vigor relation, some profound connections of cambial activity–environmental condition were determined. Five out of the six investigated varieties expressed cambial activity precisely dictated by the drought/rainy episodes, indicating their adaptive potential. Thus, miniature rose breeding goals can be set not only for vigor reduction but for different environments. Future study needs to focus on the reciprocal crossing combinations to reveal the type of vessel size inheritance.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10090996/s1, Table S1: Correlation analysis of morphological, anatomical, and histological parameters in the investigated rose cultivars.

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