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Anatomical characteristics of *Prunus domestica* vascular tissue and their implications for selection programmes

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Abstract

Knowledge of varieties' morphological and anatomical characteristics could assist in cultivar selection aimed at higher production, better fruit quality and lower orchard maintenance costs. The aim of the study was to evaluate anatomical characteristics of vascular tissue of shoots, petioles and leaf main veins of plum (*Prunus domestica* L.) cultivars with a particular emphasis on xylem and vessel hydraulic properties. The results singled out the cultivars with the most desirable combination of anatomical characters in terms of moderate vegetative growth, high yield and good fruit quality ('Toptaste' followed by 'Čačanska lepotica'). A possible application of xylem parameters as preselection criteria in early vegetative period was evaluated, and the link between anatomical properties, tree vigour, cropping potential and fruit quality was discussed. Anatomical traits, especially vessel lumen area, vessel frequency and distribution of vessels in size classes, affect vegetative growth as well as fruit production and quality, since they jointly affect water conductivity and vulnerability to cavitation and drought. Lower shoot vessel lumen values and more numerous small-sized vessels indicated lower vegetative growth, whilst higher vessel lumen values in petiole and lamina induced better lamina photosynthetic tissue water supply, which increased productivity and fruit quality parameters.

Obtaining information about the key anatomical traits at early vegetative stage could help breeders to predict the ultimate tree vigour.

Keywords: plum, xylem, hydraulic conductivity, shoot anatomy, leaf anatomy, yield, fruit quality.

Introduction

Plum (*Prunus domestica* L.) selection programmes are mostly directed toward development of new cultivars with enhanced fruit quality as well as resistance to low temperatures, frosts, drought, Plum pox virus and fungal diseases but also aim to provide cultivars with a wide-pyramidal, moderately dense crown with flexible branches (Milenković et al., 2006). In the selection aimed at fruit quality, the goal is to achieve high and regular production of table cultivars characterized by large, blue skinned fruits with firm, juicy mesocarp. In fruit trees, vegetative and fruit growth are in direct competition, whereby less vigorous trees tend to have higher fruit load and *vice versa* (Peschiutta et al., 2013). Reduced fruit tree growth is a desirable characteristic from the cost and orchard maintenance perspective and can be induced by altered water transport (Trifiló et al., 2007). Since xylem is the vascular tissue responsible for water transport, relationships between xylem and fruit tree characteristics have been extensively studied (Gonçalves et al., 2007; Tombesi et al., 2010; 2011; Zorić et al., 2012; Peschiutta et al., 2013). *Prunus* genotypes differ in their morphological and physiological traits and show great variability in fruit morphological parameters, which provides important starting point for use of these genotypes in future breeding programmes (Nisar et al., 2015).

Root and stem xylem anatomical traits, especially vessel diameter and frequency, exert high influence on hydraulic conductance, which is proportional to the sum of all vessel diameters raised to the power of four (Schuldt et al., 2013). Vessel lumen area is the largest and hydraulic conductivity is the highest in strong roots; it decreases in trunks and is the lowest in terminal branches (Gonçalves et al., 2007). Xylem anatomy and that of roots in particular is strongly related to tree vigour, as more vigorous trees tend to have a greater proportion of vessels in larger size classes. In turn, this results in better water transport, which among *Prunus* species was confirmed for peach and cherry trees (Tombesi et al., 2010; 2011; Ljubojević et al., 2013; 2017). Some authors postulate that root and rootstock as well as shoot xylem

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anatomical characteristics of young peach and cherry trees, especially vessel number and diameter, could be used as preselection parameters, when size reduction is the primary objective (Gonçalves et al., 2007; Tombesi et al., 2011; Zorić et al., 2012).

Grouped vessels may provide alternative pathways for water transport in case of vessel embolism by bypassing through functional vessels from the same group (Zhao, 2016). On the other hand, vessel grouping increases vulnerability to embolism due to the air aspiration through the pits. Embolism reduces hydraulic conductivity by decreasing the number of active xylem elements (Ogasa et al., 2013). Wood density is positively correlated with cavitation resistance, since vessels with larger lumen are more susceptible to cavitation. On the other hand, they transport water more efficiently pointing to a significant trade-off between resistance to cavitation and water transport efficiency. Vulnerability index (VI) represents the ratio of vessel diameter and frequency suggesting biological safety of vessels (Scholz et al., 2013). Lower indices characterize xerophytic taxa with numerous narrow-lumen vessels, which is a physiologically safer trait, as it restricts air embolism. Therefore, higher hydraulic safety is associated with hydraulic conductivity loss (Gonçalves et al., 2007). Lens et al. (2011) found that xylem conductivity in *Acer* species was positively correlated with vulnerability index as a result of vessel diameter relationship, while exhibiting negative correlation with the vessel grouping index (VGI).

In *Prunus* species, until maturation, fruit growth is governed by the amount of water fruit receives from the tree (Peschiutta et al., 2013). A result of phloem flow of carbohydrates is fruit size, which is always positive, and xylem water flow, which can be both positive and negative. In *P. persica*, as large quantities of water are being transported from tree to fruit, fruit growth is sustained by both phloem and xylem with 30% and 70% relative contribution, respectively (Morandi et al., 2007). Therefore, greater understanding of vascular tissue characteristics could also assist in the improvement of fruit production and quality.

In the present study, detailed anatomical analysis of terminal shoots, leaf petioles and main veins of four *Prunus* cultivars of different growth and cropping potential was performed. The aim was to evaluate vascular tissue anatomical characteristics with a particular emphasis on xylem and vessel hydraulic properties, in order to: (1) ascertain if these traits varied across the studied cultivars; (2) study the link between anatomical properties, tree vigour, cropping potential and fruit quality; (3) evaluate a possible application of xylem parameters as preselection criteria in early vegetative trait evaluation; and (4) select cultivars with the most desirable anatomical traits in terms of moderate vegetative growth, high yield and good fruit quality.

Materials and methods

As a part of the present study, the samples of shoots analysed were taken from the experimental orchard in 2018 at Rimski Šančevi Experimental Farm (average annual temperature 11.3°C, average annual rainfall 583 mm, altitude 84 m a.s.l.), Faculty of Agriculture, University of Novi Sad, Northern Serbia. This was a one-year experiment, since the primary goal was to make comparison between the cultivars, which were grown under the same environmental conditions. Two-year-old plum trees with lateral shoots grafted on the rootstock Wawit were planted in spring of 2012 ensuring 4 m spacing between the rows and 2 m distance between trees within each row. For every cultivar, a minimum of 30 trees were planted. Experimental trees were trained as spindles. The orchard was covered with anti-hail nets, and irrigation was applied. Clean strips were maintained under trees by contact herbicides, whereas alleys between rows were covered with grass. Fertilizers were applied according to the analysis of soil (chernozem) (according to WRB, 2015). Spraying treatments against pests and diseases were applied according to the recommendations pertaining to commercial orchards.

For the experiment, four plum (*Prunus domestica* L.) cultivars were selected. 'Čačanska lepotica' is characterized by a moderately vigorous tree and loose pyramidal crown of high yielding capacity. This self-fertile cultivar is a regular and heavy bearer under all agroecological conditions and on all rootstocks (Popara et al., 2020). 'Čačanska rodna' has a moderately vigorous tree, medium-dense, pyramidal to wide-pyramidal crown and supple branches that rarely break under crop load. It has very small leaves on overcropping trees and is characterized by outstanding cropping potential. This self-fertile cultivar yields small- to medium-sized fruit (Glišić et al., 2016). 'Presenta' is characterized by slow growing, moderately vigorous trees with loose crown. It is self-fertile with high and regular cropping potential and yields fruit of medium size (Hartmann, 1998). 'Toptaste' has a moderately vigorous tree with upright growth habit. It is self-fertile with high and early production potential and wide harvest window yielding medium-sized fruit (Mühlenz, Schwizer, 2013).

For anatomical analysis, 10 one-year-old shoots were sampled from 10 trees of each cultivar in dormancy (BBCH scale 00). Segments of shoots were fixed and preserved in ethanol:glycerine:water fixative at the 4:1:5 ratio. Cross-sections were obtained using hand microtome and razor blade and were analysed under the light microscope. For anatomical analysis of petiole and the main vein, 10 leaves were sampled from 10 trees of each cultivar. Leaves were taken from current-year shoots (BBCH scale 39) positioned in the upper part (at a height >1.5 m) of the selected trees and were fixed and preserved in 50% ethanol. Cross-sections were obtained using cryostat Leica CM 1850 (Germany) at cutting temperature −20°C and section thickness 40 μm. Measurements were performed using Image Analysis System Motic Plus (Motic, Germany). Vascular tissue proportions were calculated in relation to the total cross-section area of the studied plant part. Percentage of vessels (%V) was determined in total xylem area. The measurements of shoot xylem and vessels were performed on three radial segments (120° apart) on each cross-section, after which mean values were calculated. All vessels in each field of vision were measured and divided into three size classes based on their cross-section lumen area. For shoots, those were: I – <300 μm^2, II – 300–500 μm^2 and III – >500 μm^2; for petioles and main veins, which had smaller vessels, the ranges were: I – <100 μm^2, II – 100–300 μm^2 and III – >300 μm^2.

The number of vessels in each group was expressed as a percentage of the total number of vessels. The vessel grouping index (VGI) was calculated as the number of all vessels per mm^2 divided by the sum of the number of solitary vessels and the number of vessel groups per mm^2. Theoretical axial hydraulic conductance (k_h) value was calculated according to the equation below based on Hagen-Poisseuille's law (Scholz et al., 2013):

$$k_h = \frac{\pi \times \rho}{128 \times \eta} \sum_{i=1}^{n} d_i^4,$$

where ρ represents fluid density (assumed to be 10^3 kg m^{-3} for water at 20°C), d is vessel diameter in meters, η denotes viscosity (assumed to be 1.002 10^{-9} MPa s for water at 20°C). Since vessels did not have circular cross-section, an idealized vessel diameter (d) was calculated from vessel lumen (VL) area: VL = $\pi d^2/4$.

Vessel frequency (VF) was calculated as the number (N) of vessels per mm^{-2}. Vulnerability index (VI) was calculated as the ratio of vessel diameter and frequency (Scholz et al., 2013).

Trunk diameter of 10 trees per cultivar was measured at the beginning of each vegetation during five years (2014–2019) using digital calipers 10 cm above graft union, and from these measurements trunk cross-section area (TCSA) was calculated. In order to exclude a part of year-to-year variations that occurred due to frost incidence, data on yield and fruit characteristics were calculated for a 5-year period. At harvest time, all fruits were collected from individual trees in order to measure total yield per tree for each year as well as calculate cumulative yield for the 5-year period. A random sample of 10 fruits was taken from 10 trees for fruit weight and total soluble solids (TSS) measurements in the same years, and the average for the 5-year period was calculated. TSS was measured using a digital refractometer RA-250 HE (Schmidt Haensch, Japan).

Data were statistically processed using software *Statistica*, version 13 (TIBCO Software Inc., USA). Analysis of variance (ANOVA) was performed, and means, coefficients of variation and correlation coefficients were calculated. The significance of differences in measured parameters between the cultivars was determined using Duncan's multiple range test ($p \leq 0.05$). Auto-correlated variables were excluded from subsequent statistical analysis.

Results

Anatomy of terminal shoots. The microscopic analysis results revealed that the plum terminal shoot cross-section area was the largest in 'Čačanska lepotica' and the smallest in 'Toptaste' (Table 1).

Table 1. Characteristics of shoot vascular tissues of *Prunus* cultivars

	'Čačanska lepotica'		'Čačanska rodna'		'Presenta'		'Toptaste'	
Cross-section area mm^2	31.2 a	26.0	29.1 ab	22.3	25.2 ab	24.2	23.2 b	24.3
Secondary phloem %	18.2 b	15.2	18.7 b	7.7	18.6 b	7.3	22.6 a	10.9
Secondary xylem %	55.2 a	13.9	52.6 a	14.4	57.9 a	6.8	51.9 a	12.1
Xylem to phloem ratio	3.1 a	19.0	2.9 a	21.1	3.1 a	10.3	2.3 b	21.3
Vessel frequency (VF) N mm^{-2}	273 bc	11.4	328 a	9.8	242 c	24.1	292 ab	15.4
Vessel lumen (VL) area μm^2	387.0 b	13.4	481.8 a	7.5	436.4 a	14.5	308.8 c	20.7
Percentage of vessels (%V)	11.0 b	8.4	15.6 a	11.7	11.3 b	15.5	10.0 b	16.8
%V <300 μm^2	25.6 b	60.0	14.5 b	45.3	22.1 b	67.8	54.9 a	34.4
%V 300–500 μm^2	55.7 a	21.3	48.6 ab	10.4	43.2 bc	21.5	35.6 c	35.7
%V >500 μm^2	18.7 b	72.3	37.0 a	21.6	34.7 a	43.2	9.5 b	84.4
Vessel grouping index (VGI)	1.22 a	6.1	1.23 a	5.1	1.15 b	5.9	1.23 a	7.4
Vulnerability index (VI)	0.082 b	16.0	0.076 b	10.1	0.103 a	26.1	0.070 b	25.3

Note. Means in the rows marked with the same letter do not differ significantly at $p \leq 0.05$ (Duncan test), coefficient of variation.

The proportion of secondary xylem did not vary significantly among the studied cultivars. The proportion of secondary phloem was significantly higher and consequently xylem to phloem ratio lower in 'Toptaste', compared to the other cultivars. Vessel analysis revealed that they were solitary or in groups arranged in a radial pattern (Figure 1).

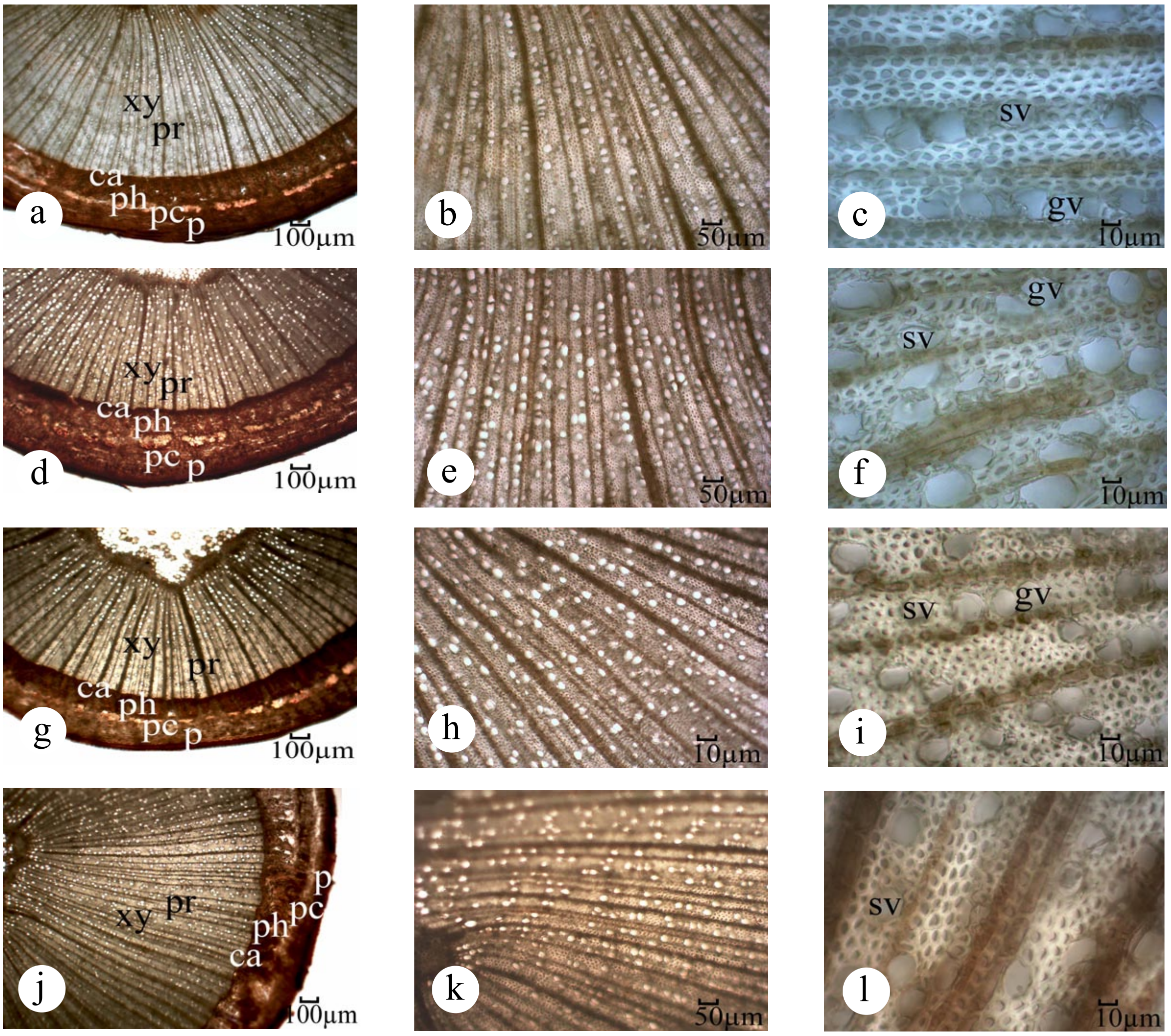
Solitary vessels were 3 times more numerous than grouped vessels, and their total percentage in xylem was consequently 2–3 times higher (Table 2).

The vessel grouping index (VGI) ranged from 1.15 to 1.23 and was not significantly correlated with theoretical axial hydraulic conductance (k_h) value. Significantly the lowest values were obtained in 'Presenta' (Table 1), although grouped vessels were most commonly arranged in pairs, and up to seven vessels could form a single group (2.2–2.3 on average). The average number of vessel groups per mm^2 of xylem area ranged from 28 in 'Presenta' to 53 in 'Čačanska rodna'. The vessel lumen area was either slightly greater in solitary vessels or was similar to that measured for grouped vessels.

When cultivars were compared with respect to vessel characteristics, the findings revealed that 'Čačanska rodna' had the highest vessel frequency of large-lumen vessels and the highest number of grouped vessels. Hence, in this cultivar, the highest total proportion of both solitary and grouped vessels was found (Tables 1 and 2). 'Toptaste' was characterized by vessels with the smallest lumen (VL mostly below 300 μm^2), which implied the lowest total percentage of vessels (%V) in xylem. 'Presenta' had large vessels of low vessel frequency and the lowest number of grouped vessels, which resulted in the lowest VGI and the highest vulnerability index. For 'Čačanska lepotica', intermediate values were obtained for most of the measured parameters leading to low k_h value. According to the coefficient of variation values, all vessel parameters showed low variability. The only exception was vessel distribution in lumen size groups, which was highly variable in all cultivars. Xylem was significantly better developed in wider shoots ($r = 0.64$). The vessel lumen was also positively correlated with shoot cross-section area ($r = 0.52$) and xylem percentage ($r = 0.33$) implying that wider shoots had larger vessels but not necessarily higher vessel frequency. Vessel lumen and vessel frequency were significantly negatively correlated ($r = -0.48$) only in solitary vessels.

Comparison of xylem and vessel parameters between the late wood and early wood zones showed that late vessels had significantly larger vessel lumen in all cultivars except 'Toptaste' but were generally not more numerous (data not shown). Consequently, total proportion of both solitary and grouped vessels was higher in late xylem. 'Čačanska rodna' was the only cultivar with consistently the highest proportion of vessels with no significant differences between the late and the early wood except in vessel lumen.

Theoretical axial hydraulic conductance (k_h) value of terminal shoots calculated per visual field of their cross-sections was significantly the highest in 'Čačanska rodna'. The lowest values were recorded for 'Toptaste', where high variability in k_h value was noted, and for 'Čačanska lepotica'. The k_h value was significantly higher in late xylem areas in 'Čačanska lepotica' and 'Čačanska rodna', whilst higher but not significantly in the other two cultivars (data not shown). Besides its positive correlation with all parameters related to the vessel lumen area, k_h value also positively correlated ($r = 0.37$) with vessel frequency. Surprisingly, vulnerability index (VI) was not significantly correlated with k_h value. It was negatively correlated ($r = -0.49$) with VGI, since grouped vessels usually had smaller vessel lumen. No correlation was established between VGI and k_h values either, whereas



a–c – 'Čačanska lepotica', d–f – 'Čačanska rodna', g–i – 'Presenta', j–l – 'Toptaste' under magnification of 40×, 100× and 400×; *ca* – cambium, *gv* – grouped vessels, *p* – periderm, *pc* – primary cortex, *ph* – secondary phloem, *pr* – parenchyma ray, *sv* – solitary vessel, *xy* – secondary xylem

Figure 1. Shoot cross-sections and a detailed view of secondary xylem of *Prunus* cultivars

Table 2. Characteristics of shoot xylem anatomy of *Prunus* cultivars

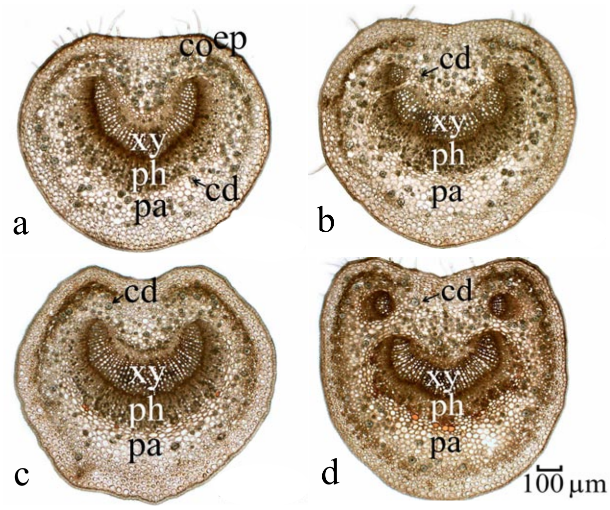
	'Čačanska lepotica'		'Čačanska rodna'		'Presenta'		'Toptaste'	
Number of solitary vessels mm^{-2}	194 b	13.7	215 ab	13.7	195 b	13.3	226 a	17.4
Lumen c-s area, solitary vessels μm^2	390.8 b	14.2	466.7 a	7.7	451.9 a	17.8	305.6 c	17.2
Solitary vessels lumen in xylem %	7.5 c	9.8	10.0 a	17.3	8.7 b	13.5	6.7 c	15.9
Number of grouped vessels mm^{-2}	90 a	25.8	114 a	24.9	62 b	48.1	103 a	38.6
Number of vessel groups, mm^{-2} of xylem	40 b	20.6	53 a	25.3	28 c	47.4	43 ab	34.3
Average number of vessels per group	2.2 a	8.3	2.2 a	8.7	2.2 a	9.6	2.3 a	8.0
Lumen c-s area of group vessels μm^2	383.1 b	13.9	496.9 a	9.2	421.0 b	14.1	312.0 c	24.7
Group vessels lumen in xylem %	3.5 b	24.7	5.6 a	23.6	2.6 b	44.9	3.3 b	31.3
Theoretical axial hydraulic conductance (k_h), 10^{-6} kg m MPa^{-1} s^{-1}	0.111 c	19.3	0.197 a	15.2	0.153 b	35.5	0.108 c	56.0

Explanation under Table 1

VI exhibited significant positive correlation with xylem percentage (r = 0.41). 'Presenta' had significantly the highest value of VI.

Petiole anatomy. In all studied cultivars, petiole was of similar cross-section area (Figure 2, Table 3).

Arc-shaped vascular tissues were accompanied by sclerenchyma fibres. Although xylem proportion did not vary significantly among the studied cultivars, the highest xylem to phloem ratio was recorded in 'Toptaste'. 'Čačanska rodna' had the highest proportion of phloem as well as low fibre content, whereas 'Čačanska lepotica' had the highest proportion of fibres. Most of the vessel characteristics did not vary significantly across the studied cultivars. 'Čačanska rodna' could be singled out as the cultivar with the lowest vessel frequency and the largest petiole vessels. Conversely, 'Čačanska lepotica' had the highest vessel frequency and vessels with the smallest lumen. Large vessels were also observed in 'Toptaste', which had the highest total percentage of vessels (%V) in xylem. The highest k_h values were obtained for 'Čačanska rodna' and 'Toptaste' and were associated with the highest VI value. 'Čačanska lepotica' had the lowest k_h value as well as VI one.



cd – crystal druses, *co* – collenchyma, *ep* – epidermis, *pa* – parenchyma, *ph* – phloem, *xy* – xylem

Figure 2. Petiole cross-sections of *Prunus* cultivars: 'Čačanska lepotica' (a), 'Čačanska rodna' (b), 'Presenta' (c) and 'Toptaste' (d)

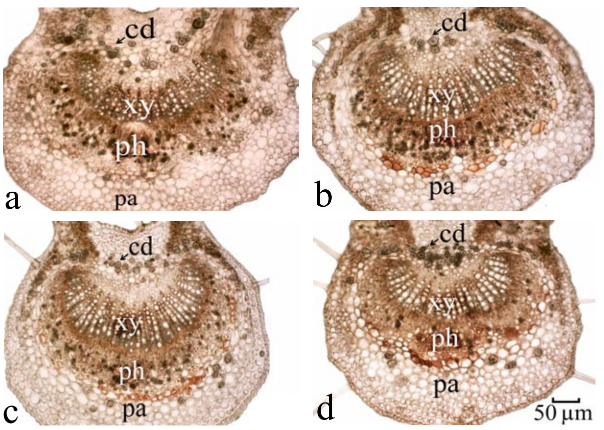
Percentage of xylem was significantly negatively correlated (r = −0.37) with the petiole cross-section area. Vessel lumen and vessel frequency were strongly negatively correlated (r = −0.69), which implied significant negative correlation between vessel frequency and k_h value (r = −0.52). Highly positive correlation was recorded between k_h and VI (r = 0.72) values. Vessel lumen area and thus vessel distribution across size classes was highly variable in all cultivars.

Main vein. All studied cultivars were characterized by similar size of the main vein cross-section area as well as phloem tissue and fibre percentage (Figure 3, Table 4). Xylem was best developed in 'Presenta', whilst being weakly developed in 'Čačanska lepotica'. Phloem proportion (%) was very variable across specimens obtained from the same cultivar, thus xylem to phloem ratio differences among the all analysed cultivars were not significant. Vessels were the most numerous in 'Čačanska lepotica' and 'Presenta'. As these cultivars also had the smallest vessel lumen, this resulted in significantly the lowest VI value. 'Toptaste' had the lowest vessel frequency but very large vessel lumen. 'Čačanska rodna' had the vessels characterized by the largest vessel lumen, 22.0% of which exceeded 300 µm². Consequently, k_h value in the main vein was significantly

Table 3. Characteristics of petiole vascular tissue of *Prunus* cultivars

	'Čačanska lepotica'		'Čačanska rodna'		'Presenta'		'Toptaste'	
Cross-section area mm²	3.13 a	14.3	2.82 a	9.9	3.13 a	14.5	3.05 a	14.2
Phloem %	12.9 b	12.9	15.1 a	9.4	12.3 b	11.5	10.2 c	9.7
Xylem %	8.88 a	8.2	9.55 a	8.4	9.74 a	9.0	9.70 a	17.3
Xylem to phloem ratio	0.70 c	13.6	0.64 c	7.8	0.79 b	10.9	0.95 a	11.5
Phloem fibres %	0.46 a	47.9	0.26 bc	22.3	0.22 c	35.1	0.35 ab	25.9
Xylem fibres %	1.78 a	14.9	1.35 b	14.4	1.38 b	11.5	1.47 b	13.4
Vessel frequency (VF) N mm⁻²	1801 a	17.1	1474 b	13.7	1586 ab	15.6	1607 ab	13.8
Vessel lumen (VL) area µm²	143.8 b	71.4	186.6 a	87.2	154.7 ab	80.8	185.8 a	81.4
Percentage of vessels (%V)	27.7 ab	18.7	28.2 ab	6.4	25.5 b	12.5	30.6 a	9.2
%V <100 µm²	43.9 a	40.0	45.8 a	26.1	47.3 a	18.2	41.4 a	26.1
%V 100–300 µm²	45.4 a	29.5	29.8 b	37.7	38.2 ab	31.5	36.3 ab	26.9
%V >300 µm²	10.7 c	107.5	24.4 a	29.0	14.6 bc	60.2	22.3 ab	40.4
Theoretical axial hydraulic conductance (k_h), 10^{-6} kg m MPa^{-1} s^{-1}	0.0163 b	42.3	0.0262 a	11.3	0.0185 b	22.0	0.0269 a	24.8
Vulnerability index (VI)	0.008 b	28.2	0.011 a	20.3	0.009 ab	22.7	0.010 ab	23.7

Explanation under Table 1



cd – crystal druses, *pa* – parenchyma, *ph* – phloem, *xy* – xylem

Figure 3. Main vein cross-sections of *Prunus* cultivars: 'Čačanska lepotica' (a), 'Čačanska rodna' (b), 'Presenta' (c) and 'Toptaste' (d)

higher in 'Čačanska rodna' than in all the other cultivars, which had similar values. The main vein cross-section area positively correlated with the %V in xylem (r = 0.51), and the %V larger than 300 µm² (r = 0.41) but also with the k_h value (r = 0.46). A significant negative correlation was recorded between vessel frequency and vessel lumen (r = −0.58).

Tree vigour, yield and fruit quality. Analysis of the trunk cross-section area (TCSA) revealed significant differences between all cultivars except between 'Čačanska rodna' and 'Toptaste'. Six years after planting, 'Čačanska lepotic' had the smallest and 'Presenta' the largest trunk diameter (Table 5).

Cultivars could also be differentiated in terms of yield. 'Toptaste' proved to be the most productive with a cumulative yield of 63.45 kg tree^{-1} followed by 'Čačanska rodna' and 'Čačanska lepotica', while 'Presenta' had significantly lower productivity (23.57 kg tree^{-1}). The cultivar with the lowest productivity also had the highest yield variability according to the coefficient of variation values. Yields were correlated with TCSA, whereby 'Toptaste' emerged as the most productive followed by 'Čačanska lepotica' and 'Čačanska rodna'. Fruit weight also exhibited large differences among the studied cultivars and was most variable in 'Čačanska lepotica' and 'Čačanska rodna'; again, 'Toptaste' had the highest values followed by 'Čačanska lepotica'. 'Toptaste' fruits also had the highest total soluble solids (TSS) content.

Table 4. Characteristics of main vein vascular tissue of *Prunus* cultivars

	'Čačanska lepotica'		'Čačanska rodna'		'Presenta'		'Toptaste'	
Cross-section area mm^2	0.795 a	22.8	0.732 ab	23.0	0.612 b	20.4	0.592 b	29.2
Phloem %	11.9 ab	52.1	9.3 b	14.1	13.7 a	32.1	11.2 ab	20.6
Xylem %	9.8 c	13.6	10.9 b	8.5	12.0 a	8.2	10.5 bc	9.7
Xylem to phloem ratio	1.1 a	55.7	1.2 a	12.8	0.93 a	21.1	0.98 a	26.1
Phloem fibres %	3.5 a	32.0	4.3 a	28.8	3.7 a	15.3	4.0 a	22.6
Xylem fibres %	2.3 b	25.8	2.7 ab	12.4	3.1 a	21.8	3.1 a	15.6
Vessel frequency (VF) N mm^{-2}	2050 a	18.2	1710 b	9.8	1979 a	11.8	1437 c	20.5
Vessel lumen (VL) area µm^2	154.7 bc	16.4	208.8 a	16.4	143.4 c	17.2	182.2 ab	20.7
Percentage of vessels (%V)	29.3 ab	17.3	32.0 a	16.1	25.8 bc	9.9	24.7 c	15.7
%V <100 µm^2	38.0 a	34.5	24.1 b	33.4	35.3 a	28.6	23.6 b	56.3
%V 100–300 µm^2	51.4 b	31.8	53.9 ab	24.5	59.7 ab	13.0	64.8 a	18.7
%V >300 µm^2	10.5 b	95.1	22.0 a	46.8	5.1 b	102.6	11.6 b	105.2
Theoretical axial hydraulic conductance (k_h), 10^{-6} kg m MPa^{-1} s^{-1}	0.020 b	34.6	0.0274 a	31.5	0.0146 b	22.4	0.0127 b	31.3
Vulnerability index (VI)	0.007 b	22.9	0.010 a	14.0	0.007 b	18.2	0.011 a	26.2

Explanation under Table 1

Table 5. *Prunus* tree growth, productivity and fruit quality (2014–2019)

Cultivar	TCSA cm^2 (in 2018)		Cumulative yield kg tree^{-1}		Yield efficiency		Fruit weight g		TSS %	
Čačanska lepotica	60.11 c	7.2	46.62 b	9.7	0.77 b	11.3	36.30 a	11.5	14.90 b	9.3
Čačanska rodna	66.89 b	4.9	49.34 b	7.9	0.73 b	7.2	29.48 b	11.4	18.37 b	11.9
Presenta	79.53 a	6.9	23.57 c	26.4	0.29 c	27.2	25.82 b	9.7	18.27 b	17.5
Toptaste	67.24 b	6.0	63.45 a	11.9	0.94 a	12.4	40.98 a	8.2	22.26 a	11.7

Explanation under Table 1

Discussion

Prunus cultivars exhibited anatomical variability at both stem and leaf level as well as in fruit productivity and quality, which reflects genetic differences among them. Differences in studied anatomical parameters among the cultivars were only quantitative and determined by genotype, since all cultivars were grown under the same conditions. Variation of quantitative parameters can be used as a starting point in breeding programmes aiming at formation of less vigorous trees. Xylem anatomical traits proved to be good predictors of tree growth rate in *Prunus* species, which might be used in programmes directed towards selection of less vigorous cultivars (Trifiló et al., 2007; Zorić et al., 2012). In our experiment, the connection between tree vigour and anatomical traits was also proved. The most vigorous cultivar 'Presenta' had large-lumen shoot vessels of significantly the lowest frequency followed by medium to high theoretical axial hydraulic conductance (k_h) value. This cultivar had the highest shoot vulnerability index (VI), and, therefore, is more susceptible to cavitation and drought. On the other hand, the least vigorous cultivar among the studied ones was 'Čačanska lepotica', which had significantly the lowest TCSA. At the same time, this cultivar was characterized by the lowest k_h value in petiole and shoot due to low vessel lumen value in petiole and medium to low in shoot followed by the highest vessel frequency in petiole and the main vein and medium vessel frequency in shoot. Shoot vessel lumen and xylem proportion positively correlated with shoot cross-section area, and shoot xylem and vessel parameters proved to affect the ultimate tree vigour more than xylem characteristics of leaves.

Hydraulic conductivity and ultimate tree vigour could not be predicted solely by the shoot xylem to phloem ratio, as was proposed by some authors for cherry trees, as xylem elements other than those conducting water (mechanical and parenchyma cells) do not affect tree vigour or fruit production (Zorić et al., 2012). Xylem to phloem ratio did not vary significantly among the studied cultivars. In shoot samples it was the lowest in 'Toptaste', whilst the petiole of the same cultivar had the highest xylem to phloem ratio.

Ultimate tree vigour in *Prunus* species could be predicted on the basis of xylem anatomy (Gonçalves et al., 2007; Tombesi et al., 2011; Zorić et al., 2012). In order to select less vigorous phenotypes, selection should be directed towards *Prunus* cultivars with small-lumen vessels in reasonable limits without negatively affecting other biological processes as well as low conductivity and cavitation susceptibility. The most desirable combination of shoot characters with respect to the dwarfing potential was found in 'Toptaste', as its vessels had the smallest lumen area, <300 µm^2 in 55% of analysed samples. In this cultivar, only 9.5% of shoot vessels were larger than 500 µm^2, which is a very good trait, since vigorous trees tend to have a greater proportion of vessels in larger size classes and, therefore, better water transport. This cultivar also had the lowest shoot xylem to phloem ratio and k_h and VI values. Similar values were recorded in 'Čačanska lepotica' in both petiole and the main vein, which also had low shoot and petiole k_h values. 'Toptaste' and 'Čačanska rodna' had medium vigour, compared to the other two cultivars. Tree vigour is also influenced by several factors like local climate, soil type, fruit species and rootstock (Donadio et al., 2019). In the first years after planting, tree vigour may be influenced by other factors such as nursery stock quality, particular requirements of the cultivar for tree training and level of yields but also rootstock characteristics (Wertheim, 1998; Blažek, Pištěková, 2012).

'Čačanska rodna' and 'Toptaste' had a high percentage of large-lumen vessels and high k_h value in both petiole and the main vein, which enabled fast and abundant water supply to lamina, the part of the leaf where photosynthetic tissue is concentrated. Productivity is also associated with plant's ability to supply water to leaves, because leaf hydration plays a key role in phloem transport and photosynthetic capacity (Sellin et al., 2008; Petit, Crivellaro, 2014). We assume that these anatomical traits were among the parameters that induced the highest cumulative yield, yield efficiency and fruit weight in 'Toptaste'. The recorded distribution of small-sized vessels in shoot and large-sized vessels in leaves of 'Toptaste' could be a very positive characteristic, because it could induce lower vigour, while enabling high leaf water supply, which could positively affect photosynthesis, organic matter production and phloem transport from leaves to fruits.

In *Prunus*, a significant correlation was previously reported between vulnerability to cavitation and drought tolerance and habitat preferences, whereby more vulnerable species were restricted to wet habitats (Cochard et al., 2008). In the present study, the highest vulnerability of shoots was noted in 'Presenta', whilst the other cultivars had safer shoot water flow systems. For 'Toptaste', the lowest shoot vulnerability to cavitation was calculated. Combination of more numerous narrow-lumen vessels induces higher hydraulic safety associated with hydraulic conductivity loss, which is a desirable trait in selection for less vigorous trees. In 'Toptaste' and 'Čačanska lepotica', it was followed by low k_h value, whilst in 'Čačanska rodna' by high k_h value due to high vessel frequency. Petioles are less vulnerable to cavitation in *Prunus laurocerasus*, *Betula*, *Juglans* and *Alnus* species but are equally resistant or more vulnerable in *Quercus* species, compared to stem (Nardini et al., 2001).

In the studied cultivars, VI values were approximately 10 times lower in petiole and the main vein, compared to shoots. VI values in petiole were similar between the cultivars but higher in 'Toptaste' and 'Čačanska lepotica' in the main vein. As leaves are easily replaced every season, leaf embolism is not as harmful to the plant as is embolism in the shoots. Moreover, hydraulic system disfunction in leaves during fruit formation could enhance water delivery from shoots to fruits. Peschiutta et al. (2013) hypothesized that water instead of moving into the leaves is delivered directly to the fruits, which thus become the main water transport sink. Phloem proportions in the main vein were not significantly different across the studied cultivars. Peschiutta et al. (2013) found that cherry cultivars with high leaf vulnerability to cavitation and low water conductivity and sap flow had low vegetative growth but larger fruit production, compared to the cultivars with the highest k_h value, resistance to cavitation and water use. Our findings for 'Toptaste' are in accordance with those reported by Peschiutta et al. (2013), although its vegetative growth was not reduced as would have been expected.

Vessel diameter is usually in close negative relationship with vessel frequency, but in positive relationship with hydraulic conductivity (Sellin et al., 2008). In the studied cultivars, vessel lumen area was in significant negative correlation with vessel frequency in both petiole and the main vein. In shoots, such correlation was obtained for solitary vessels only. Solitary vessels are 3 times more numerous with 2–3 times higher total percentage in xylem but not necessarily larger, compared to grouped vessels, and, therefore, expected to play a much more prominent role in water transport efficiency. Consequently, in our experiment, no relationship was established between theoretically calculated VGI and k_h values, whereas negative correlation was calculated between VI and VGI values. Lens et al. (2011) provided the first empirical test of correlation between VGI and k_h values reporting negative correlation in *Acer* species. Among the traits that were associated with enhanced cavitation resistance and lower conductivity were wood density and vessel grouping index (VGI) as well as vessel diameter, which was negatively correlated with these parameters. Using xylem model, Loepfe et al. (2007) demonstrated that hydraulic conductivity and vulnerability to embolism increase with xylem network connectivity, i.e., vessel grouping. They thus proposed that connected vascular elements are more available for water transport but also for spread of embolism, further suggesting that connectivity, conduit size and inter-conduit resistance are the key hydraulic conductivity delimiting factors. However, the proposed model is overly simplistic, since communication among vessels, water and air bubble transport also occur through the pits. Thus, xylem cannot be represented as the sum of its conduits, which explains misalignment between the results obtained in this work and those reported by Loepfe et al. (2007).

The average number of vessels per group (2.2–2.3) did not vary significantly across the analysed cultivars and was similar to the one recorded in *Betula platyphylla* (2.1) (Zhao, 2016). Species from dry habitats showed a higher degree of vessel grouping, less solitary vessels, greater maximum vessel sizes and an increase of the percentage of grouped vessels with increasing vessel size (von Arx et al., 2013). In line with these assertions, the studied cultivars are better adapted to more humid habitats.

Vegetative growth competes with reproductive sinks, and lower vigour usually positively affects fruit production (Peschiutta et al., 2013). This proved to be true for 'Toptaste', the cultivar of medium vigour but high yield of large-sized fruits. Among the studied cultivars, 'Toptaste' proved to be the most productive, yielding the best fruit size, yield and TSS, which is in accordance with the findings reported by Blažek and Pištěková (2012). In contrast, the most vigorous cultivar 'Presenta' had the lowest yield and yield to TCSA ratio. This cultivar was previously described as moderately vigorous, which was proved in our experiment, although was the most vigorous among the tested cultivars.

Tree vigour can be modified by altered water transport, which depends largely on xylem anatomical properties. Anatomical parameters that proved to affect vegetative growth of *Prunus* cultivars the most were vessel lumen area as well as distribution of vessels in size groups followed by vessel frequency, which jointly affected water conductivity and vulnerability to cavitation and drought. Lower shoot vessel lumen values and more numerous small-sized vessels induced lower vegetative growth. On the other hand, higher vessel lumen values in petiole and lamina induced better lamina photosynthetic tissue water supply, which increased productivity and fruit quality parameters.

Since these results were obtained from one-year experiment, they can be useful for comparison of the studied cultivars grown under the same growing conditions and as preliminary data for further experiments directed towards selection and breeding of less vigorous *Prunus* cultivars of high productivity. Xylem parameters of terminal shoot parts could be used as preselection criteria in early vegetative trait evaluation. Obtaining information about the key anatomical traits, which affect vegetative growth like vessel size and frequency parameters, at this early vegetative stage could help breeders to predict the ultimate tree vigour. Selection of cultivars with a desirable combination of anatomical traits could change plant growth in a desirable way towards obtaining less vigorous trees.

Conclusions

1. Variation of xylem quantitative parameters can be used in breeding programs aiming at formation of less vigorous *Prunus* trees. Xylem parameters of terminal shoot parts could be used as preselection criteria in early vegetative trait evaluation.

2. Some anatomical traits like vessel lumen (VL) area, vessel frequency (VF) and distribution of vessels in size-classes may affect vegetative growth as well as fruit production and quality. Lower shoot VL values and more numerous small-sized vessels induced lower vegetative growth, water conductivity and vulnerability to cavitation.

3. The most desirable combination of anatomical traits in respect to the dwarfing potential and fruit productivity was found in 'Toptaste' followed by 'Čačanska lepotica'.

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Prunus domestica apytakos audinio anatominės savybės ir jų reikšmė selekcijos programoms

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Santrauka

Žinios apie morfologines ir anatomines veislių savybes galėtų padėti jas atrinkti, siekiant didesnio derliaus, geresnės vaisių kokybės ir mažesnių sodo priežiūros išlaidų. Tyrimo tikslas – įvertinti naminės slyvos (*Prunus domestica* L.) veislių ūglių, lapkočių ir lapų pagrindinių gyslų audinio anatomines savybes, ypač ksilemos ir vandens indų hidraulines savybes. Tyrimo rezultatai leido išskirti veisles, pasižyminčias geriausiu anatominių požymių deriniu su vidutiniu vegetatyviniu augimu, dideliu derliumi ir gera vaisių kokybe ('Toptaste', po jos 'Čačanska lepotica'). Buvo įvertinta galimybė taikyti ksilemos parametrus kaip išankstinės atrankos kriterijus ankstyvuoju vegetacijos laikotarpiu ir nustatytas ryšys tarp anatominių savybių, medžių gyvybingumo, derliaus potencialo ir vaisių kokybės. Anatominiai požymiai, ypač indų vidinės erdvės dydis, indų dažnumas ir jų pasiskirstymas pagal dydžių klases turi įtakos vegetatyviniam augimui, taip pat vaisių derliui ir kokybei, nes kartu daro įtaką vandens laidumui ir pažeidžiamumui dėl kavitacijos bei sausros. Mažesnė ūglių indų vidinė erdvė ir didesnis mažų indų skaičius rodė prastesnį vegetatyvinį augimą, o didesnė indų vidinė erdvė lapkočiuose ir lapalakščiuose lėmė geresnį lapalakščių fotosintetinio audinio aprūpinimą vandeniu; tai padidino ūglių produktyvumą ir vaisių kokybės parametrus.
Gauta informacija apie pagrindinius anatominius požymius ankstyvuoju vegetacijos laikotarpiu galėtų padėti selekcininkams numatyti slyvų medžių gyvybingumą.

Reikšminiai žodžiai: derlius, hidraulinis laidumas, ksilema, lapų anatomija, slyva, ūglių anatomija, vaisių kokybė.