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Anatomical Characteristics of Cherry Rootstocks as Possible Preselecting Tools for Prediction of Tree Vigor

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Abstract An anatomical study of roots and stems of five self-rooted cherry rootstocks with different growth control potentials was performed to compare their structure and xylem anatomy. The aim was to correlate anatomical parameters with rootstock dwarfing potential and theoretical hydraulic conductance (k_h), and to evaluate the potential application of anatomical characteristics in the preselection process for prediction of ultimate tree vigor. One of the mechanisms of water transport efficiency reduction in dwarfing rootstock stems is from the rootstock xylem anatomy. Anatomical parameters of 'Gisela 5' and 'Mazzard' were typical for dwarfing and vigorous rootstocks, respectively, and were thus suggested as reference rootstocks. Significantly greater vessel diameter and frequency were found in invigorating and dwarfing rootstocks, respectively. Higher k_h was obtained in roots, compared to stems, due to significantly larger vascular elements. Dwarfing rootstocks had lower k_h due to small vessel lumens and percentage and, to a lesser extent, because of low wood/cortex ratios or percentage of wood. A higher percentage of wood or xylem in cherry roots and stems was not always positively correlated with their conductivity and vigor. Thus, these parameters cannot be reliably used in prediction of the ultimate vigor, although this method was previously suggested for some other fruit tree species. The most reliable anatomical parameters for that purpose proved to be vessel frequency, vessel lumen area, and percentage of vessels on wood cross section. These characteristics could thus be an effective way to estimate dwarfing capacity and could be applied in rootstock selection and breeding programs.

Keywords Hydraulic conductivity · Prunus · Root anatomy · Stem anatomy · Xylem · Vessels

Introduction

The use of rootstocks with different size-controlling potential has become very common in fruit tree production worldwide (Trifilò and others 2007; Tombesi and others 2010a). The selection of dwarfing rootstocks of fruit crops has received great attention in the past as well as in recent years. Reduced vegetative growth is a desirable feature that contributes to higher productivity and more cost-effective orchard maintenance (Seleznyova and others 2008; Fassio and others 2009). Dwarfing rootstocks also reduce labor costs and pesticide usage, while increasing precocity and fruit quality (Olmstead and others 2004). Knowledge of the biological properties of rootstocks is essential in understanding the mechanisms by which rootstock affects scion growth.

A general trend of increasing root and stem vessel diameter and decreasing vessel density with tree height was observed across and within fruit and forest tree species (Castro-Diez and others 1998; Zach and others 2010). In many tree species, xylem characteristics of root and stem (xylem-to-phloem ratio, vessel density, and size) significantly affect rootstock growth potential (Castro-Diez and others 1998; Meland and others 2007; Trifilò and others 2007). These anatomical traits play a crucial role in root

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and stem hydraulic conductance, which is proportional to the sum of the vessel diameters, whereby each term is raised to the power of four (Tyree and Ewers 1991; Sellin and others 2008; Végvári and others 2008; Tombesi and others 2010a; Zach and others 2010). Therefore, vessel number and diameter are the main determinants of hydraulic conductance, which theoretically could be calculated using the Poiseuille-Hagen equation (Tyree and Ewers 1991). In dwarfing apple and peach rootstocks, hydraulic conductance was lower when compared to vigorous or semivigorous rootstocks (Basile and others 2003; Atkinson and others 2003). The presence of smaller and fewer vessels in the scion and graft union of cherry trees could contribute to lower hydraulic resistance and reduced scion growth (Olmstead and others 2006). McElrone and others (2004) consider roots, especially its vessels, as the best source of hydraulic differences within and among species.

The mechanisms by which a rootstock is dwarfed are complex and poorly understood. It is strongly believed that the main mechanisms that control dwarfing are correlated with rootstock anatomical properties, mainly the characteristics of wood and xylem conduits. Anatomical characteristics should be the starting point in understanding the biological basis of dwarfing. Several studies have been published concerning the anatomy of apple and peach dwarfing rootstocks (Beakbane and Thompson 1947; Miller and others 1961; Vercambre and others 2002; Atkinson and others 2003; Basile and others 2003). Lower xylem-to-phloem ratio, smaller vessels, and lower hydraulic conductance were characteristics of apple dwarfing rootstocks. However, little data on the anatomy of cherry rootstocks and their connection with dwarfing have been found. It is still unclear whether parameters that were used for apple trees, such as xylem-to-phloem ratio, or xylem anatomical characteristics could be applied successfully as preselection criteria for dwarfing cherry trees. Meland and others (2007) found that the total area of functional xylem in rootstock shanks, scion stem, and graft union was larger in vigorous (‘Colt’) compared to dwarfing rootstock (‘Gisela 5’). Cherry trees grafted on dwarfing rootstock ‘Gisela 5’ had higher vessel frequency and lower vessel diameter, hydraulic conductivity, and vulnerability index (vessel diameter/vessel frequency ratio) compared to the trees on the invigorating rootstocks (Goncalves and others 2007). These authors analyzed only a few anatomical parameters on two commercial rootstocks. To the best of our knowledge, more extensive anatomical analyses of cherry rootstocks have not been conducted. Therefore, in the present study we performed a detailed anatomical analysis of roots and stems using five self-rooted cherry rootstocks with different growth control potentials (Blažkova 2004; Olmstead and others 2006; Tomaszewska and Nychnerewicz 2006; Meland and others 2007; Goncalves and others 2007; Long and Kaiser 2010). ‘Mazzard’ rootstock is the same species as sweet cherry (*Prunus avium*); thus, it is compatible with all sweet cherry scion cultivars. It has high vigor (125% compared to ‘Colt’) and moderate productivity. Slightly less vigorous (110% compared to ‘Colt’) is ‘Mahaleb’ (*P. mahaleb*), one of the most drought-tolerant cherry rootstocks (Long and Kaiser 2010). Although ‘Colt’ (*P. avium* × *P. pseudocerasus*) was released as a semidwarfing rootstock, in irrigated orchards the tree becomes vigorous and similar in size to ‘Mazzard’ (Meland and others 2007; Long and Kaiser 2010). One of the most dwarfing but very productive rootstocks is ‘Gisela 5’ (*P. cerasus* × *P. canescens*), reducing vigor by up to 45% compared to ‘Colt’ (Meland and others 2007; Long and Kaiser 2010). ‘PHL-A’ (*P. avium* × *P. cerasus*) is also a dwarfing rootstock, slightly more vigorous than ‘Gisela 5’ (50% compared to ‘Colt’) but highly resistant to winter frosts (Blažkova 2004).

The main objectives of this study were to (1) test the correlation between root and stem anatomical properties (secondary xylem characteristics in particular); (2) establish a link between root and stem anatomical characteristics and cherry rootstock dwarfing potential and theoretical hydraulic conductance; and (3) evaluate the possible application of xylem anatomical characteristics to the preselection process for predicting the ultimate vigor that a cherry rootstock will impart to a scion.

Material and Methods

The anatomical study was carried out on five ungrafted, contrasting cherry rootstocks. The selected trees were grown at the research plots of the Faculty of Agriculture, University of Novi Sad. ‘Mazzard’ rootstock (*P. avium*) and ‘Mahaleb’ (*P. mahaleb*) had been sexually propagated by sowing seeds taken from one individual tree of each type. The ‘Mazzard’ tree was open-pollinated and the ‘Mahaleb’ self-pollinated selection. Both rootstock progenies expressed a high level of uniformity in seedling vigor and plant morphology. ‘Gisela 5’, ‘Colt’, and ‘PHL-A’ were propagated by softwood cuttings taken from 4-year-old virus-free mother trees. All rootstocks were managed similarly in the orchard and were ungrafted. Segments of second lateral roots, sampled 10 cm from the branching point and approximately 4–6 mm in diameter, and segments of 2-year-old lateral branches (at the beginning of the second vegetation), sampled at the second internode from the base and approximately 4 mm in diameter, were separated from five replicate plants per rootstock (three samples per tree) (Fig. 1). Plant material was fixed and preserved in 60% ethanol. Cross sections were made using

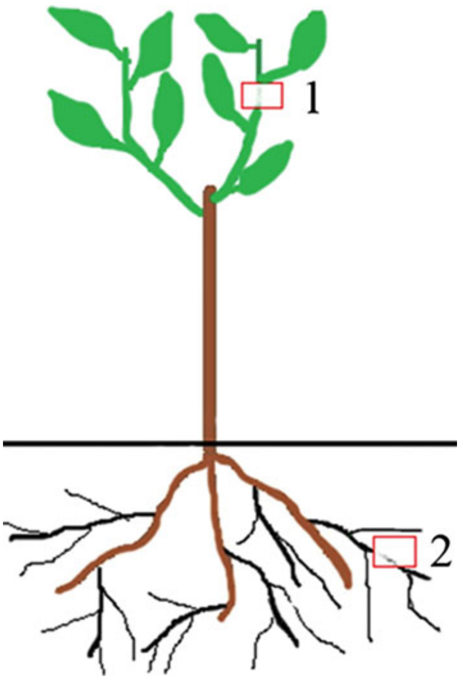


Fig. 1 Diagram representing sampling positions of root and stem segments. **1** Two-year-old lateral branches (at the beginning of the second vegetation), sampled at the second internode from the branching point. **2** Second lateral roots, sampled 10 cm from the branching point

a hand microtome and cryostat Leica CM 1850 (cutting temperature = −20°C; section thickness = 60 μm). The sections were stained for lignin using the acid phloroglucinol test (Jones and others 2003).

Cross sections were examined and measurements performed using a light microscope and Image Analyzing System Motic Images Plus. Root and stem cross-section areas were measured and relative proportions calculated for each tissue of interest. Anatomical analyses of root cross sections included measurements of proportions of periderm, secondary cortex (phloem), and secondary wood (xylem). The measurements of xylem and vessels were performed on 12 visual fields of each root cross section. Four radial segments, 90° apart, were observed in the external, middle, and internal zones, reaching from the vascular cambium (early wood) to the pith (late wood). All vessels in every field of vision were measured. On stem cross sections, proportions of periderm, primary cortex, secondary cortex (phloem), secondary wood (xylem), and primary xylem with medulla were determined. The measurements were performed on four radial segments, 90° apart, on each stem cross section.

Vessel lumen area (VL), diameter (VD), and frequency (VF, number of vessels/mm^2) were measured and calculated. As dicot wood is composed of vascular elements, wood parenchyma (axial and rays), and wood fibers (Esau 1977), the percentage of vessels (%V), rays (%R), and xylem area (%X) were calculated per wood cross-section area. Vessel diameter was calculated from the mean value of the vessel lumen area [$\mathrm{VL} = (\mathrm{VD}/2)^2\,\pi$], to estimate the idealized diameter. Based on their cross-section lumen area, root vessels were divided into three size classes: I, vessels with lumen area smaller than 700 μm^2; II, vessels with lumen area in the range 700–2,000 μm^2; and III, vessels with lumen area greater than 2,000 μm^2. Because stem vessels were significantly smaller than root vessels, they were classified, based on their lumen area, into three groups as follows: I, lumen area smaller than 300 μm^2; II, lumen area in the range 300–700 μm^2; and III, lumen area greater than 700 μm^2. The number of vessels in each group, expressed as a percentage of the total number of vessels (100%), was calculated.

Based on anatomical measurements, the theoretical axial hydraulic conductance (k_h) of roots and stems was calculated according to the formula given by Tyree and Ewers (1991), based on Hagen-Poisseuille's law:

$$k_\mathrm{h} = \frac{\pi \cdot \rho}{128 \cdot \eta} \sum_{i=1}^{n} d_i^4$$

where d is the diameter of the vessels in meters, ρ is the fluid density (assumed to be 10^3 kg m^{-3} for water at 20°C), and η was the viscosity (assumed to be 1.002×10^{-9} MPa s for water at 20°C).

Data were statistically processed by analysis of variance, and means, standard errors, and correlation coefficients were calculated using STATISTICA for Windows version 10.0 (StatSoft, Tulsa, OK). The significance of differences in measured parameters between the samples was determined using Duncan's multiple range test ($P \le 0.05$). The general structure of sample variability was established by principal component analysis (PCA) based on a correlation matrix. Multivariate discriminant function analysis (MDA) was conducted to test the pattern of sample grouping based on the selected quantitative anatomical characteristics of their roots and stems.

Results

Anatomical Properties of Roots from Ungrafted Rootstocks with Different Size-Controlling Capacities

Anatomical analyses showed significant differences in root anatomical parameters among the analyzed rootstocks (Fig. 2). Roots of dwarfing 'PHL-A' had the smallest percentage of secondary wood (xylem), the highest percentage of secondary cortex (phloem), and the lowest wood/cortex ratio (1.07) (Table 1). Invigorating rootstocks

'Mazzard' and 'Mahaleb' had the highest wood/cortex ratio (2.23 and 1.89, respectively). Percentage of periderm was also higher in the vigorous rootstocks. Vessel frequency (VF) was significantly the highest and vessel size significantly the smallest in 'Gisela 5'. The majority of these vessels (77.9%) were smaller than 700 μm^2 and none exceeded 2,000 μm^2. 'PHL-A' had VF and VL similar to those of the vigorous rootstocks, that is, a low but uniform number of relatively large vessels. 'Mahaleb' had the lowest VF, but its vessels were the largest. According to their size, most of the root vessels of vigorous rootstocks belonged to group II, followed by group III.

High percentages of xylem per wood cross section (%X) and consequently low percentages of rays (%R) were recorded for dwarfing 'Gisela 5' as well as for vigorous 'Mazzard' and 'Mahaleb'. However, the lowest vessel area to wood cross-section ratios (%V) were also recorded in 'Gisela 5'.

Generally, a significant negative correlation of VF was found with VL ($r = -0.80$) and the size of the root cross section ($r = -0.66$), implying that wider roots did not necessarily have more vessels. Overall, the root cross-section size was significantly positively correlated with VL ($r = 0.66$) and %V ($r = 0.48$) but negatively correlated with percentage of vessels smaller than 700 μm^2 ($r = -0.70$).

Comparison of xylem and vessel parameters among the three examined wood zones in root cross sections (external-younger, middle, and internal-older) showed that differences were not statistically significant in most of the samples (Table 2). The vessels were significantly more numerous in the older wood zone in 'Colt' and 'Mahaleb', but their size did not vary significantly with age, except in 'Mahaleb' and 'Gisela 5', where the younger vessels were significantly smaller. %V was significantly higher in older wood in 'Colt', 'Mahaleb', and 'Gisela 5'. %R and %X did not vary significantly within wood zones. Guided by these results and comparisons of the three root wood zones, further analyses were performed on mean values of examined parameters only.

According to the PCA analysis results, the most variable among the examined root parameters were VF, VL, and %V in the first size class (Table 3). These parameters loaded on the first principal component axis and contributed to the total variation by 38.9%. The second principal component accounted for a further 31.0% of the total variance and was defined by the percentages of secondary wood and cortex, as well as their ratio. The scatterplot showed clear separation and

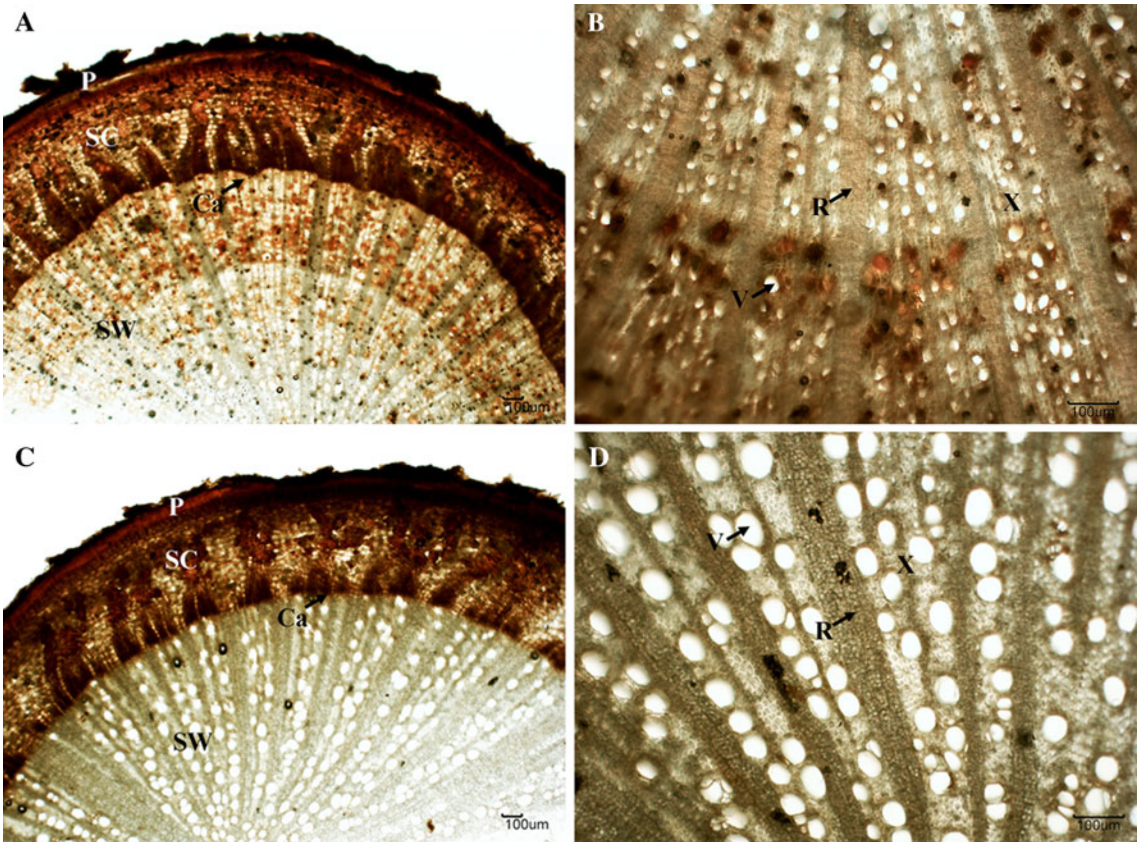


Fig. 2 Light micrographs of root cross sections of contrasting rootstocks. **a**, **b** 'Gisela 5'. **c**, **d** 'Mazzard'. *P* periderm; *SC* secondary cortex; *SW* secondary wood; *Ca* cambium; *V* vessels; *R* rays; *X* xylem

Table 1 Root anatomical characteristics of five cherry rootstocks with different growth control potentials (mean values)

	Mazzard	Mahaleb	Colt	PHL-A	Gisela 5
% Periderm	10.9^{a}	10.0^{a}	6.6^{b}	6.0^{b}	8.8^{ab}
% Secondary cortex	28.9^{c}	31.5^{c}	40.3^{ab}	45.7^{a}	37.7^{b}
% Secondary wood	60.1^{a}	58.3^{a}	53.0^{ab}	48.3^{b}	53.5^{ab}
Wood/cortex ratio	2.23^{a}	1.89^{ab}	1.35^{c}	1.07^{c}	1.42^{bc}
%X	78.3^{a}	76.4^{a}	73.0^{b}	75.3^{ab}	78.5^{a}
%V	12.0^{a}	9.2^{ab}	11.5^{a}	9.4^{ab}	7.2^{b}
%R	21.7^{b}	23.7^{b}	27.0^{a}	24.7^{ab}	21.5^{b}
VF (*N*/mm^{2})	89.6^{b}	64.4^{c}	89.0^{b}	78.8^{b}	155^{a}
VL (µm^{2})	1,416^{a}	1,528^{a}	1,362^{a}	1,229^{a}	520^{b}
VD (µm)	41.8^{a}	42.8^{a}	40.9^{a}	39.2^{a}	25.2^{b}
%V $<$ 700 µm^{2}	12.5^{b}	16.6^{b}	17.8^{b}	10.4^{b}	77.9^{a}
%V 700–2,000 µm^{2}	68.9^{ab}	56.8^{b}	62.8^{b}	79.5^{a}	22.1^{c}
%V $>$ 2,000 µm^{2}	18.6^{a}	26.6^{a}	19.3^{a}	10.1^{ab}	0^{b}

$^{a,\ b,\ c}$ Differences among the means designated with the same letter were not significant, according to Duncan's test, at $P < 0.05$

% Periderm, *% secondary cortex*, *% secondary wood* proportions of the root cross-section; *%X* percentage of xylem area per wood cross section; *%V* percentage of vessels per wood cross section; *%R* percentage of rays per wood cross section; *VF* vessel frequency; *VL* vessel lumen area, *VD* vessel diameter; %V $<$ 700 µm^{2} = vessels with lumen area smaller than 700 µm^{2}; %V 700–2,000 µm^{2} = vessels with lumen area 700–2,000 µm^{2}; %V $>$ 2,000 µm^{2} = vessels with lumen area greater than 2,000 µm^{2}

Table 2 Anatomical characteristics of vessels and xylem in the external, middle, and internal root wood zones of cherry rootstocks (mean values)

	Mazzard	Mahaleb	Colt	PHL-A	Gisela 5
VF ext	85.8^{a}	56.8^{b}	70.1^{b}	68.9^{b}	156^{a}
VF mid	85.2^{a}	54.0^{b}	81.4^{b}	80.3^{a}	153^{a}
VF int	97.7^{a}	82.3^{a}	116^{a}	87.4^{a}	155^{a}
VL ext	1,399^{a}	1,134^{b}	1,404^{a}	1,356^{a}	370^{b}
VL mid	1,519^{a}	1,756^{a}	1,411^{a}	1,226^{a}	487^{ab}
VL int	1,330^{a}	1,695^{a}	1,272^{a}	1,104^{a}	704^{a}
%X ext	78.6^{a}	77.8^{a}	70.3^{a}	76.2^{a}	79.0^{a}
%X mid	77.2^{a}	75.2^{a}	73.5^{a}	74.4^{a}	78.8^{a}
%X int	79.1^{a}	76.1^{a}	75.1^{a}	75.3^{a}	77.8^{a}
%V ext	11.3^{a}	6.6^{b}	8.7^{b}	9.1^{a}	5.4^{b}
%V mid	12.5^{a}	9.5^{a}	11.9^{ab}	9.6^{a}	6.6^{b}
%V int	12.1^{a}	11.5^{a}	13.9^{a}	9.4^{a}	9.7^{a}
%R ext	21.4^{a}	22.2^{a}	29.7^{a}	23.8^{a}	21.0^{a}
%R mid	22.8^{a}	24.8^{a}	26.5^{a}	25.6^{a}	21.2^{a}
%R int	20.9^{a}	23.9^{a}	24.9^{a}	24.7^{a}	22.2^{a}

$^{a,\ b}$ Differences among the means of the three zones designated with the same letter were not significant for the rootstock, according to Duncan's test, at $P < 0.05$

VF vessel frequency; *VL* vessel lumen area; *%X* percentage of xylem area per wood cross section; *%V* percentage of vessels per wood cross section; *%R* percentage of rays per wood cross section; *ext* external root wood zone; *mid* middle root wood zone; *int* internal root wood zone

high homogeneity of 'Gisela 5' specimens along the first axis (Fig. 3). Other rootstocks were more heterogeneous and no other distinct clusters could be recognized. Most of the 'Mazzard' and 'Mahaleb' specimens clustered along the second axis, based on the similar variability of percentages of secondary cortex and wood.

Anatomical Properties of Stems from Ungrafted Rootstocks with Different Size-Controlling Capacities

The stem anatomical analysis results showed that 'PHL-A' and 'Mazzard' had the highest percentage of primary cortex and wood with medulla, respectively (Table 4).

Table 3 Principal component analysis of root anatomical parameters

Anatomical trait	Factor 1	Factor 2	Factor 3
% Periderm	0.1770	−0.5422	−0.5465
% Secondary cortex	−0.3580	0.8885*	−0.0400
% Secondary wood	0.3494	−0.8073*	0.2860
Wood/cortex ratio	0.2901	−0.8965*	0.1694
VF	0.7643*	0.3616	−0.4022
VL	−0.8719*	−0.4309	−0.1703
%X	0.6709	−0.4525	0.0328
%V	−0.6166	−0.3317	−0.5140
%R	−0.6709	0.4525	−0.0328
%V I group	0.8771*	0.3412	−0.2321
%V II group	−0.6657	−0.1439	0.5770
%V III group	−0.6793	−0.4586	−0.4697
Eigenvalue	4.6642	3.7224	1.4753
% Total variance explained	38.8682	31.0202	12.294

Loadings marked by an asterisk (*) are >0.7000 and significant for the axis

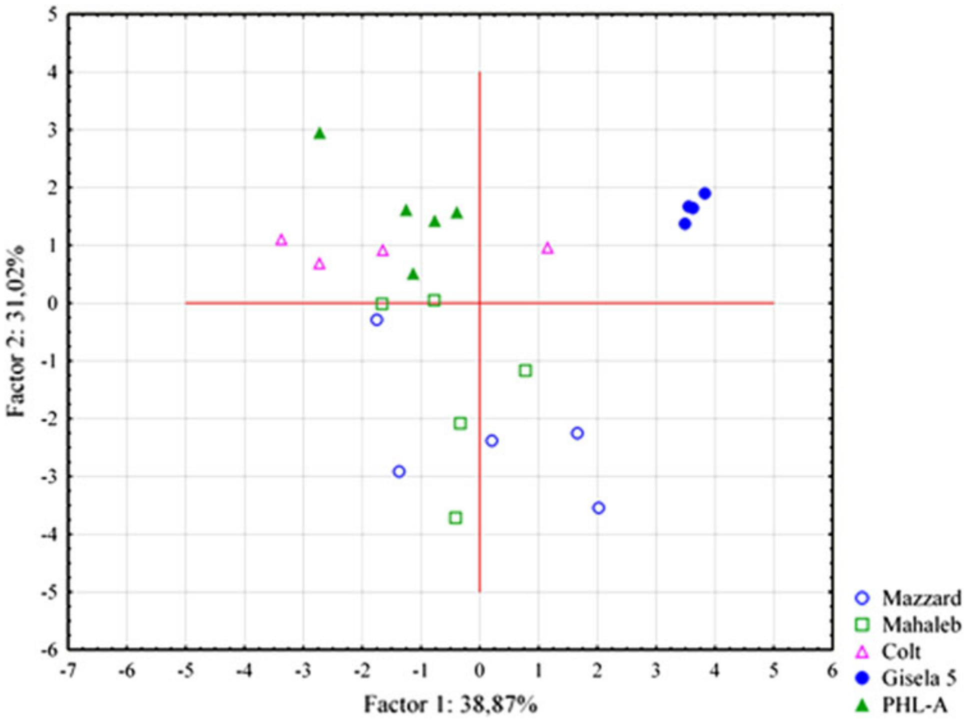
% Periderm, *% secondary cortex*, *% secondary wood* proportions of the root cross section; *%X* percentage of xylem area per wood cross section; *%V* percentage of vessels per wood cross section; *%R* percentage of rays per wood cross section; *VF* vessel frequency; *VL* vessel lumen area; %V I group = %V < 700 μm^2; %V II group = %V 700–2,000 μm^2; %V III group = %V > 2,000 μm^2

Dwarfing 'PHL-A' and, surprisingly, vigorous 'Mazzard' had significantly the smallest percentages of secondary wood and secondary wood/cortex ratios (Fig. 4). Percentages of secondary wood were higher and similar in the other three rootstocks. Stem vessel frequency varied in a relatively small range for the analyzed rootstocks, from 155 to 222 per mm^2. A combination of very high VF and small VL was recorded in dwarfing 'Gisela 5' and 'PHL-A' as well as in 'Mahaleb'. Most of the vessels were smaller than 300 μm^2 in 'Mahaleb' and 'Gisela 5' (61.2 and 55.9%, respectively), and none in 'Mahaleb' and only a few in 'Gisela 5' were larger than 700 μm^2. 'Mazzard' could be singled out as a rootstock with a high number (213/mm^2) of very large vessels (average lumen area = 617 μm^2), of which 32.7% were in the third size group. It also had significantly higher %V. However, dwarfing rootstocks had the highest %X and the lowest %R. In contrast, 'Mazzard' and 'Mahaleb' had the highest %R and the lowest %X.

Stem VF and VL were not significantly correlated, except in 'Gisela 5' ($r = -0.95$). The size of the stem cross section did not significantly affect VF. In general, the size of the vessels was significantly positively correlated with %V ($r = 0.85$) and stem cross-section area ($r = 0.69$). With increased stem size, the percentage of small vessels significantly declined ($r = -0.63$).

The PCA analysis results showed that the first principal component explained 39.2% of the total variation. It was mostly defined by the parameters related to the size of the vessels: VL, %V, and %V in groups I and III (Table 5). The second principal component explained another 25.5% of the variation. Parameters that loaded on the second axis were percentages of primary cortex, secondary wood, and wood/cortex ratio. Vessel frequency and %V in the second

Fig. 3 PCA scatterplot based on root anatomical parameters of five different cherry rootstocks. Points represent tree specimens



size class loaded on the third principal component and contributed to total variation by an additional 13.3%. All rootstocks were heterogeneous and showed high variability (Fig. 5). No specific clusters could be defined based on the PCA analysis of stem characteristics.

Theoretical hydraulic conductance (k_h), calculated per visual field of cross sections, was significantly higher in roots compared to stems (Fig. 6). The highest values in roots were obtained for ‘Mazzard’ (0.75×10^{-6} kg m MPa^{-1} s^{-1}) and the lowest for ‘Gisela 5’ (0.15×10^{-6} kg m MPa^{-1} s^{-1}), whereas other rootstocks had similar values. The k_h values in stems were highest in the vigorous rootstock ‘Mazzard’ (0.31×10^{-6} kg m MPa^{-1} s^{-1}). However, they were similar and significantly lower in other rootstocks.

Detailed analysis of all examined anatomical parameters revealed strong associations of tree vigor with vessel frequency, vessel lumen area, and percentage of vessels per wood cross section. The discriminant analysis scatterplot, obtained using these traits, showed that dwarfing ‘Gisela 5’ was completely separated along the first discriminant axis from all the other analyzed rootstocks based on the selected root and stem parameters, which contributed to discrimination on the first axis at a very high percentage of 84.5% (Table 6; Fig. 7). The second discriminant axis further separated vigorous rootstock ‘Mazzard’, mostly based on its stem vessel frequency and lumen area.

Discussion

The results of our study showed that some root anatomical parameters could be singled out as important in distinguishing between dwarfing and vigorous rootstocks. The roots of dwarfing ‘PHL-A’ had the lowest percentage of secondary wood and wood/cortex ratio, while ‘Gisela 5’ roots had the lowest percentage of vessels in xylem, the lowest vessel lumen area, and the highest vessel frequency compared to vigorous rootstocks. The results of PCA analysis confirmed that ‘Gisela 5’ samples were homogeneous and clearly separated as typical dwarfing. ‘PHL-A’ had the lowest wood/cortex ratio but similar VF, VL, and theoretical axial hydraulic conductance values to vigorous rootstocks. That might explain its better resistance to abiotic stress, especially drought, compared to dwarfing ‘Gisela 5’. However, only 10.1% of its vessels were larger than 2,000 μm^2, while in vigorous rootstocks this percentage was significantly higher (18.6–26.6%). In three of five rootstocks, root segments in the external zone had a lower number of vessels per mm^2, but their lumen was significantly smaller only in ‘Gisela 5’ and ‘Mahaleb’. This was because these segments were younger and closer to the cambium, and conduits were probably not of a definite size. Differences between the middle and internal root zones, as well as differences in total percentages of xylem and rays, were not statistically significant.

The values of %V, VL, and VF in the stem followed a similar pattern to that observed in the roots. The observed

Table 4 Stem anatomical characteristics of five cherry rootstocks with different growth control potentials (mean values)

	Mazzard	Mahaleb	Colt	PHL-A	Gisela 5
% Periderm	5.0^{ab}	6.9^{a}	4.7^{ab}	6.8^{ab}	4.5^{b}
% Primary cortex	12.3^{bc}	13.0^{bc}	15.3^{ab}	17.0^{a}	12.0^{c}
% Secondary cortex	26.0^{a}	19.4^{c}	19.3^{c}	27.0^{a}	22.7^{b}
% Secondary wood	45.8^{bc}	55.5^{a}	52.1^{ab}	41.6^{c}	55.0^{a}
% Primary wood + medulla	11.3^{a}	5.1^{b}	8.5^{ab}	7.6^{ab}	5.8^{b}
Secondary wood/cortex ratio	1.8^{b}	2.9^{a}	2.7^{a}	1.5^{b}	2.4^{a}
%X	85.5^{c}	87.1^{c}	89.4^{b}	91.9^{a}	90.7^{ab}
%V	13.0^{a}	5.6^{c}	6.5^{bc}	7.9^{b}	6.2^{bc}
%R	14.5^{a}	12.9^{a}	10.6^{b}	8.1^{c}	9.3^{b}
VF (N/mm^2)	213^{a}	189^{ab}	155^{b}	222^{a}	208^{a}
VL (μm^2)	617^{a}	310^{c}	429^{b}	373^{bc}	309^{c}
VD (μm)	27.9^{a}	19.8^{c}	23.3^{b}	21.7^{b}	19.8^{c}
%V < 300 μm^2	2.5^{c}	61.2^{a}	19.2^{bc}	35.5^{b}	55.9^{a}
%V 300–700 μm^2	64.9^{a}	38.9^{b}	75.2^{a}	61.4^{ab}	43.4^{b}
%V > 700 μm^2	32.7^{a}	0^{b}	5.5^{b}	3.1^{b}	0.7^{b}

[a, b, c] Differences among the means designated with the same letter were not significant, according to Duncan’s test, at $P < 0.05$

% Periderm, *% primary cortex*, *% secondary cortex*, *% secondary wood*, *% primary wood + medulla* proportions of the root cross section; *%X* percentage of xylem area per wood cross section; *%V* percentage of vessels per wood cross section; *%R* percentage of rays per wood cross section; *VF* vessel frequency; *VL* vessel lumen area; *VD* vessel diameter; %V < 300 μm^2 = vessels with lumen area smaller than 300 μm^2; %V 300–700 μm^2 = vessels with lumen area 300–700 μm^2; %V > 700 μm^2 = vessels with lumen area greater than 700 μm^2

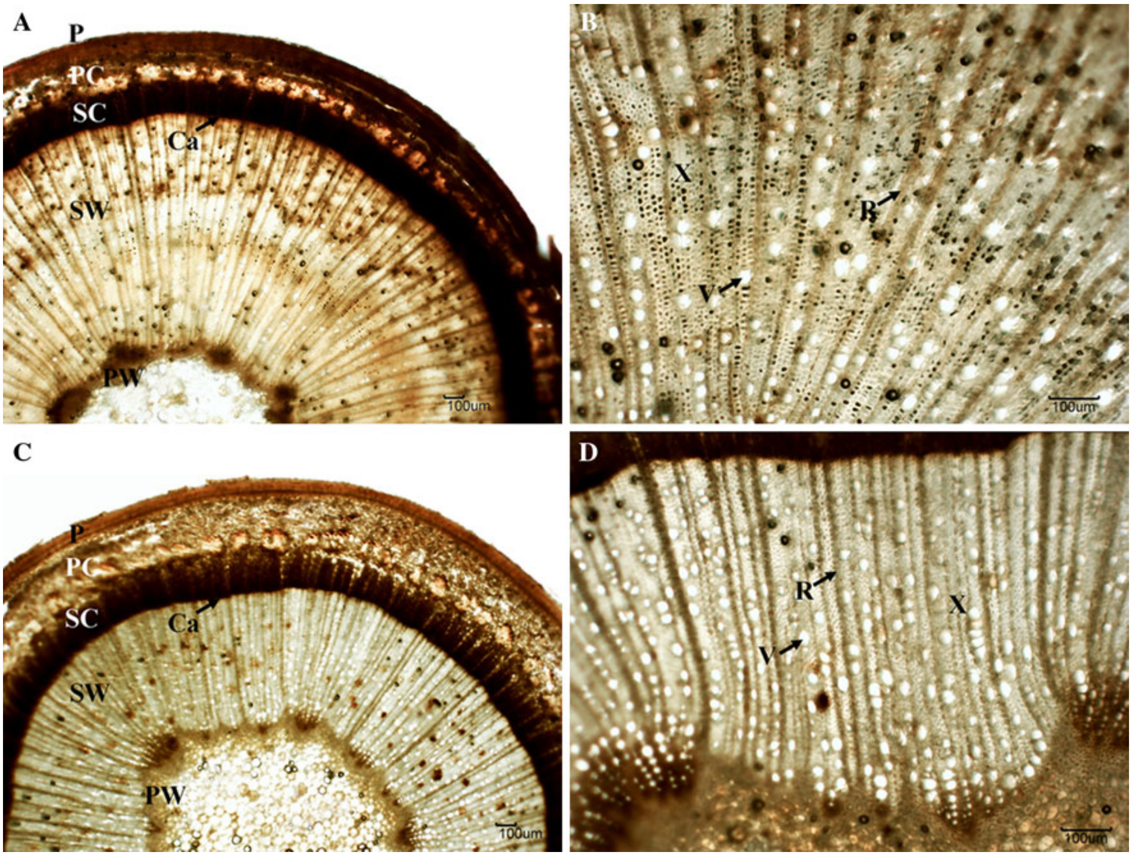


Fig. 4 Light micrographs of stem cross sections of contrasting rootstocks. **a**, **b** ‘Gisela 5’. **c**, **d** ‘Mazzard’. *P* periderm; *PC* primary cortex; *SC* secondary cortex; *SW* secondary wood; *PW* primary wood; *Ca* cambium; *V* vessels; *R* rays; *X* xylem

variation in percentages of stem tissues among the rootstocks did not conform to expectation. For example, dwarfing ‘Gisela 5’ had a higher percentage of secondary wood than vigorous rootstocks, and ‘Mazzard’ had a low secondary wood/cortex ratio. However, the apparent contradiction can be explained when analyzed in tandem with vessel characters. A larger percentage of wood or xylem does not always imply higher conductivity and higher tree vigor. Xylem is also composed of elements other than vessels (libriform, tracheids, parenchyma), that do not directly contribute to tree vigor; instead, they affect mechanical strength and tree resistance to abiotic stress. Hence, with respect to the effect on dwarfing, percentage of vessels, rather than percentage of wood or xylem, is the key parameter for comparison among the rootstocks. We proved that for rootstocks of different size-controlling potential, %V, which is directly responsible for conductivity, was of higher discerning value than %X and %R. Although vigorous ‘Mazzard’ had a low stem wood/cortex ratio, its high number (213/mm^2) of very large vessels (32.7% were larger than 700 μm^2) compensated for the low percentage of wood and xylem and induced high hydraulic conductance and intensive tree growth. Similarly, although dwarfing ‘Gisela 5’ had a high percentage of secondary wood and xylem, the percentage of vessels within was very low (6.2%), as was their lumen area, which induced lower hydraulic conductivity and dwarfing habitus.

Because all examined trees grew in the same conditions, we consider anatomical differences between the rootstocks as genetically determined. Results corresponded to those presented by Goncalves and others (2007), who showed that VD values of all organs were significantly larger in invigorating cherry plants, while VF was significantly higher in dwarfing cherry plants. Dwarfed cherry trees had significantly lower xylem/phloem ratios than invigorating trees due to their low xylem thickness. Our results are in accordance with the findings of Olmstead and others (2006) that smaller and fewer vessels in the scion contribute to reduced scion growth. Our results are also consistent with the findings of Tombesi and others (2010a) who reported that vigorous peach rootstocks had larger vessel diameters but a smaller number of vessels per visual field and that vessels were larger in roots compared to stems. A general trend of increasing vessel diameter and decreasing vessel density with tree height was observed across and within tree species (Trifilò and others 2007; Zach and others 2010).

The lumen of vessels in cherry roots and stems is generally larger for samples with wider organs, with fewer vessels per mm^2. A larger cross-section area implies a lower VF and a lower number of small vessels but a higher %V. VF and VL, as well as VF and stem cross-section area, were significantly negatively correlated in the roots but not in the stems. Goncalves and others (2007) also found a significant reverse relationship between VF and VL only in the roots of cherry rootstocks of different vigor, while there was no correlation between these two parameters in stems. The same authors observed the lowest VF in large-sized roots, which is in accordance with our results.

Significant differences in vessel size and frequency were recorded between roots and stems. Vessels in roots were, on average, from 1.7 (‘Gisela 5’) to 4.9 (‘Mahaleb’) times larger than vessels in stems, but their frequency was from 1.3 (‘Gisela 5’) to 2.9 (‘Mahaleb’) times higher in stems. According to Goncalves and others (2007), roots of cherry rootstocks had 2.7 times larger mean VD than shoot vessels. Schweingruber and others (2008) also noted that in *Prunus persica*, ring porosity with large earlywood vessels was characteristic of the root structure, whereas small earlywood vessels were typical of the stem structure. McElrone and others (2004) found that conduit diameters were smallest in stems, intermediate in shallow roots, and largest in deep roots in four tree species. Increased

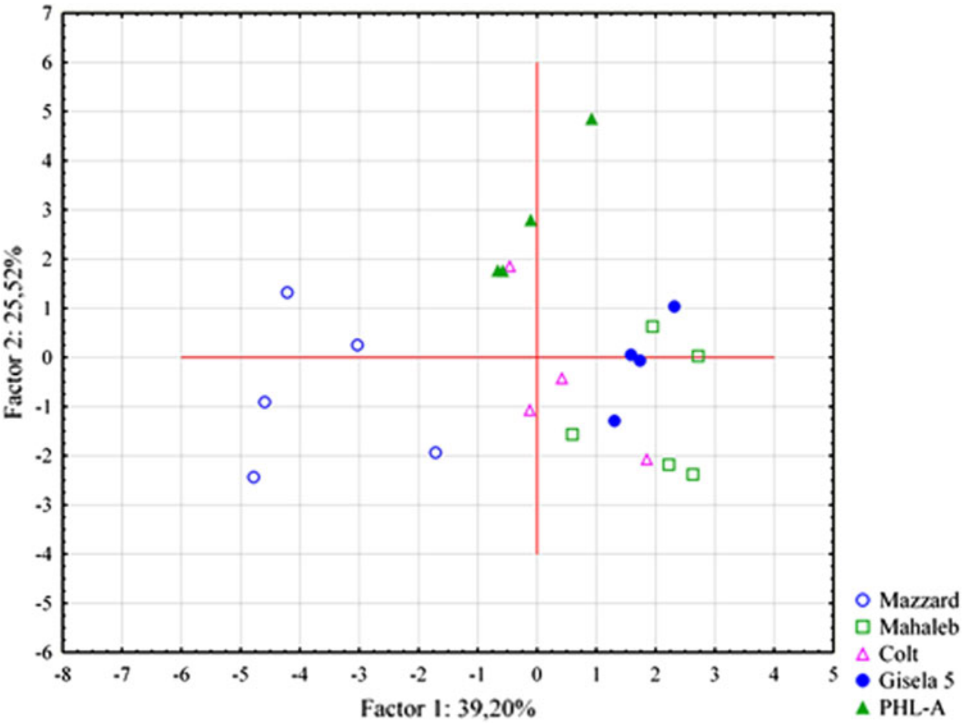
Table 5 Principal component analysis of stem anatomical parameters

Anatomical trait	Factor 1	Factor 2	Factor 3
% Periderm	0.2165	0.5202	−0.0761
% Primary cortex	−0.0025	0.7809*	0.2374
% Secondary cortex	−0.6246	0.4375	−0.2907
% Secondary wood	0.5237	−0.8096*	−0.0405
% Primary wood + medulla	−0.6030	0.3719	0.1981
Secondary wood/cortex ratio	0.6362	−0.7211*	0.1336
%X	0.5086	0.6983	0.1738
%V	−0.9295*	−0.0769	−0.3027
%R	−0.5086	−0.6983	−0.1738
VF	−0.2762	0.2557	−0.8473*
VL	−0.9375*	−0.2430	0.1541
%V I group	0.8245*	0.1317	−0.4973
%V II group	−0.5069	−0.0105	0.7018*
%V III group	−0.8578*	−0.2524	−0.1274
Eigenvalue	5.4887	3.5725	1.8552
% Total variance explained	39.2047	25.5177	13.2518

Loadings marked by an asterisk (*) are >0.7000 and are significant for the axis

% Periderm, *% primary cortex*, *% secondary cortex*, *% secondary wood*, *% primary wood + medulla* = proportions of the root cross section; *%X* percentage of xylem area per wood cross section; *%V* percentage of vessels per wood cross section; *%R* percentage of rays per wood cross section; *VF* vessel frequency; *VL* vessel lumen area; %V I group = %V < 300 μm^2; %V II group = %V 300–700 μm^2; %V III group = %V > 700 μm^2

Fig. 5 PCA scatterplot based on stem anatomical parameters of cherry rootstocks. Points represent tree specimens



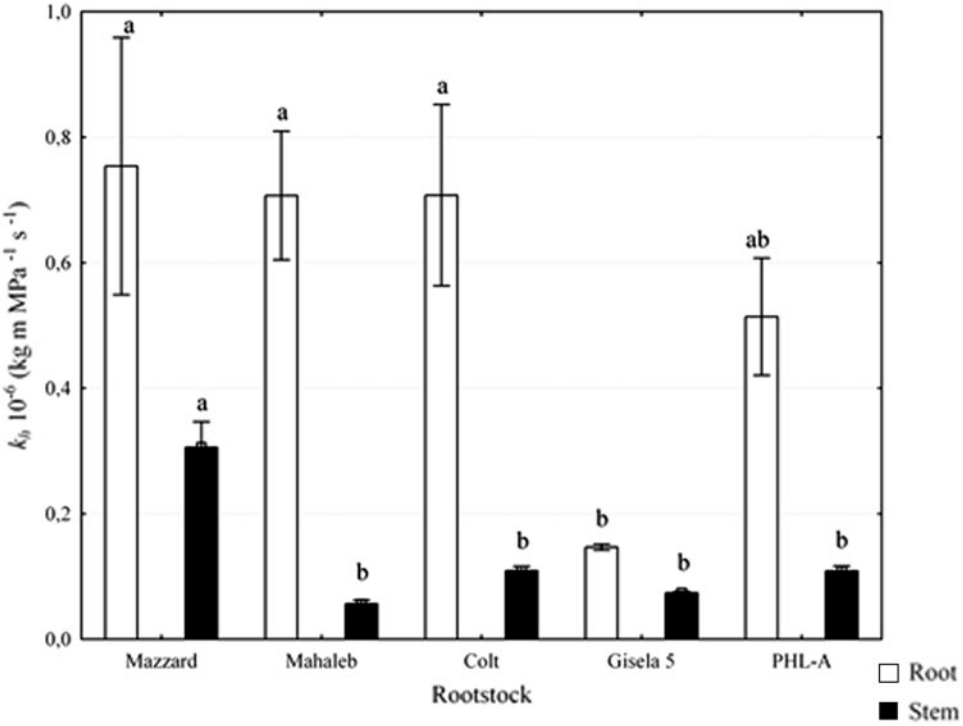


Fig. 6 Theoretical axial hydraulic conductance per visual field of root and stem cross sections of cherry rootstocks. The values represent the means of four visual fields (radial segments, 90° apart) of five sections per rootstock ± SE. Differences between the means designated with the *same letter* were not significant, according to Duncan's test, at $P < 0.05$

Table 6 Discriminant analysis based on the selected root and stem anatomical parameters

Anatomical trait	Root 1	Root 2	Root 3
Root VF	4.1734*	−0.0900	−1.2658*
Root % V	−7.9309*	−0.7060*	4.6941*
Root VL	7.7308*	0.6304	−4.6891*
Stem VF	−2.1854*	−1.0867*	4.7999*
Stem %V	1.8251*	0.6618	−4.6047*
Stem VL	−0.9932*	−1.3730*	2.5574*
Eigenvalue	52.6782	7.3577	1.7962
Cumulative proportion	0.8452	0.9632	0.9920

Loadings marked by an asterisk (*) are >0.7000 and are significant for the axis

hydraulic efficiency of roots compared to stems is also associated with higher cavitation and drought-induced embolism vulnerability (Dickison 2000; McElrone and others 2004). Larger xylem conduits are also more vulnerable to freezing and freezing-induced cavitation and provide weaker mechanical support (Ewers 1985; Castro-Diez and others 1998; Schweingruber 2007). We believe that different root and stem growth conditions, as well as different demands of their surroundings under and above the ground, induced differences in their xylem anatomy. As roots were supported by the soil and were thus less exposed to environmental stress, wide root conduits could have been developed as adaptations that contributed to better and more efficient water transport. The stem grows under higher environmental pressure (temperature changes, winds, and so on), and one of its basic functions is giving vertical support to the tree plant. Therefore, stem conduits with their smaller lumen and higher frequency, in addition to their contribution to water transport, also contributed to the higher mechanical tree strength. Stem trait differences in tissues at an early developmental stage correspond to those described for adult plants and can thus be considered as ontogenetically fixed (Castro-Diez and others 1998).

Variations in xylem conduit size radically affect the functions of the conducting system due to the fourth-power functional relationship between diameter and water flow through a capillary tube (Tyree and Ewers 1991; Castro-Diez and others 1998; McElrone and others 2004; Goncalves and others 2007). Thus, even a small increase in conduit diameter has a significant effect on hydraulic conductivity. Theoretical calculation of xylem hydraulic conductance may be an effective means to estimate the rootstock dwarfing capacity (Tombesi and others 2010a). In our research, significantly higher values of estimated k_h were obtained for roots compared to stems due to significantly larger vascular elements. The differences in measured k_h corresponded to the variation in rootstock xylem characteristics. Lower hydraulic conductance of dwarfing rootstocks (especially of 'Gisela 5') was induced mostly by small VL and lower %V, and to a lesser extent by a smaller wood/cortex ratio or smaller percentage of wood. This is in accordance with Goncalves and others (2007), who found that medium-sized cherry roots had much higher k_h (2620%) than terminal shoots and that smaller conduits of

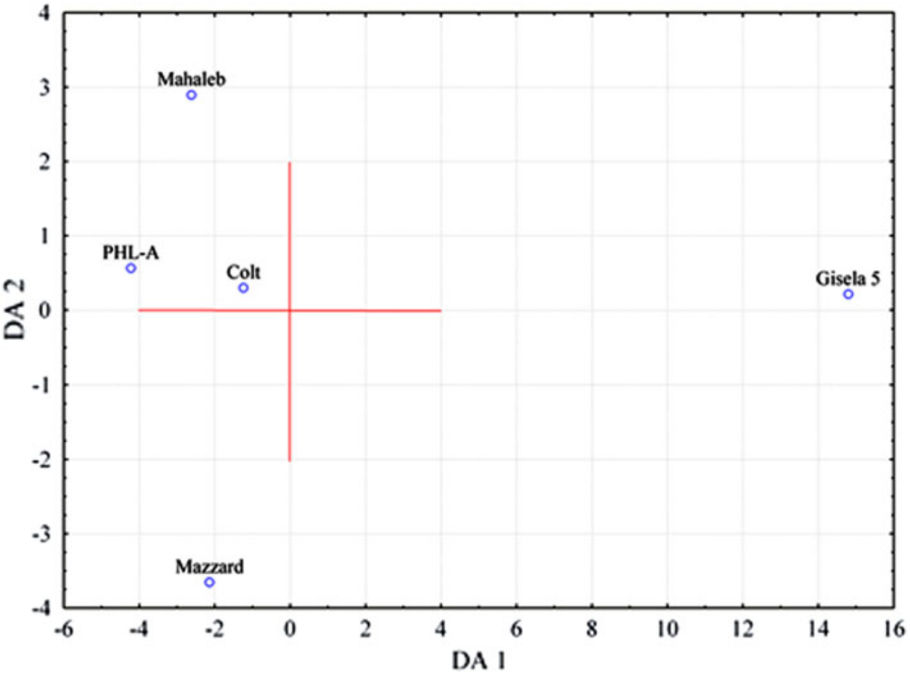


Fig. 7 Discriminant analysis scatterplot based on selected root and stem anatomical parameters of cherry rootstocks

trees on ‘Gisela 5’ had lower relative k_h. Rootstock k_h has been directly related to tree vigor in peach (Tombesi and others 2010a, b) and apple (Atkinson and others 2003). Low k_h of the rootstock decreases stem water potential, limits stomata conductance, and therefore lowers photosynthetic assimilation. In cherry, it reduces shoot extension growth rate but not the fruit size, which is genetically controlled (Lang 2011).

Only strongly vigorous (‘Mazzard’) and strongly dwarfing (‘Gisela 5’) rootstocks fit the expected pattern of variation in anatomical parameters and behaved as typical vigorous and dwarfing rootstocks, respectively, and should thus be used as referent rootstocks in future studies. Other rootstocks did not always show the expected anatomical characteristics. Although ‘Colt’ was a vigorous rootstock, its root parameter values were similar to those of dwarfing rootstocks. This could be explained by its origin; this rootstock was originally released as a semidwarfing rootstock, but in irrigated orchards it produced a vigorous tree, similar in size to ‘Mazzard’ (Long and Kaiser 2010). The ‘Colt’ vigor is variable, depending on the soil, scion cultivar, and other conditions, and in northern Europe its tree size is often approximately 30% smaller (Meland and others 2007). Clearly, its habitus is greatly influenced by environmental conditions. Although considered as a vigorous, ‘Mahaleb’ also showed a dwarfing effect on cherry scions when compared to ‘Mazzard’, and some of its characteristics could not be considered typical for vigorous trees.

Our findings suggest that cherry rootstock anatomical parameters might provide an additional explanation of the mechanisms of the rootstock effect on scion growth. We concluded that the presence of vessels with a smaller lumen area, a higher percentage of small vessels, and, consequently, a lower total percentage of vessels on cross section are the main anatomical changes in the xylem that cause lower hydraulic conductance. We share the opinion of Tombesi and others (2010a) that the number and the diameter of xylem vessels appear to be the key factors that influence dwarfing potential of rootstocks by influencing hydraulic conductance. Anatomical parameters of rootstock root conduits could be of even greater importance than that of the stem. Actually, the pathway of water flow in dwarfing rootstocks begins with a changed root anatomical structure and formation of small vessels, which have low water conductivity. As a smaller amount of water reaches the stem, its water potential consequently becomes lower, which further limits transpiration and photosynthesis and leads to the formation of dwarfing rootstock habitus. Furthermore, trees grafted on dwarfing rootstocks would have reduced growth.

Beakbane and Thompson (1947) stated that the percentage of root bark was the best single criterion to adopt when attempting to forecast the ultimate vigor of apple trees on a series of new rootstocks. Végvári and others (2008) referred to a low xylem ratio in stems of ‘Gisela 5’ as one of the main elements, together with stem porosity, that affected hydraulic conductivity. Our study did not confirm these statements in relation to cherry rootstocks. The percentages of wood and cortex did not always follow the expected pattern in vigorous and dwarfing rootstocks, especially in the stems. In our opinion, parameters such as percentage of xylem and rays, as well as stem anatomical parameters (apart from stem vessel lumen area), could not be applied successfully in the preselection process for prediction of the ultimate vigor a cherry rootstock would impart to a scion. The most reliable parameters, which might be useful in vigor prediction, proved to be vessel frequency, vessel lumen area, and percentage of vessels on wood cross section. These characteristics might be an effective way to estimate the dwarfing capacity of specific genotypes and could find a practical application in current rootstock selection and breeding programs.

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