

CORRELATIONS BETWEEN STEM ANATOMY AND GROWTH VIGOR IN SELECTED PLUM ROOTSTOCK GENOTYPES

Andaç Çavdar¹, Murat İsfendiyaroğlu²

¹ Aegean Agricultural Research Institute, Department of Horticulture, 35660, İzmir, Turkey

² Ege University, Faculty of Agriculture, Department of Horticulture, 35100, İzmir, Turkey

ABSTRACT

Turkey is particularly one of the centres of origin where many cultivars of *Prunus cerasifera* Ehrh., *P. institia* L. and *P. spinosa* L. occurred. These species have been worldwide considered with their rootstock features. In this study, correlations between plant growth vigor and structure of vascular elements in some plum rootstock genotypes were examined. For this purpose, 12 different wild *P. cerasifera* genotypes were used together with some commercially evaluated rootstocks as control. Results showed that plant elongation augmented in parallel with the increments in xylem diameter and xylem area ratio. Increases in cortex area ratio decreased the tree height. Moreover, the plant height was decreased by the elongation of xylem vessels in the vertical axis, but was increased by the decrease in xylem vessel area and the decrease in xylem vessel area increased the plant height. Comparing the entire evaluating genotypes, T7 and B9 were the most dwarf, while 17 and B6 the most vigorous once was concluded. In this study, a method which would accelerate the rootstock breeding works by using the structure of plant vascular elements, to predict the growth vigour as a pre-selection criteria which is important in plum rootstock selection was exposed.

Key words: *Prunus* spp., dwarfing, histology, cortex, xylem, vessel

INTRODUCTION

In modern fruit growing, differences between growth vigor of rootstocks are of great importance in currency. So, new dwarfing rootstocks suitable for different purposes have been improved in different countries. The purpose of researchers and nurserymen to develop the rootstocks that can be economically propagated in large areas and also have individual adaptation characteristics [Ferree 1982, Cummins and Aldwinckle 1983, Licznar-Małańczuk and Sosna 2013]. Knowing the biological features of a rootstock, is important to understand how it is affecting the growth of scion. In particular, water uptake and transport through the xylem are necessary for protecting the plant from desiccation, and to allow the attendance of photosyn-

thesis as well [Kramer and Boyer 1995]. As a matter of fact, a strong correlation was predicted between the tree height and the length of vascular elements in dwarf trees [Baas et al. 1984]. Rashedy et al. [2014], stated that the prediction of dwarfing potential of some mango (*Mangifera indica* L.) cultivars was possible by examining the stem anatomy and the ratio of xylem and phloem tissues, besides the ratio of xylem diameters in different sizes were the good indicators of dwarfing potential. In mango and olive, number of xylem vessels can be used for rating of rootstocks at the nursery stage, while the xylem vessel area, number of xylem vessel and percentage of xylem vessels having small diameter can be accepted as the good in-

dicators of dwarfing [Majumdar et al. 1972, Hegazi et al. 2013]. Majumdar et al. [1972] also predicted that cortex/xylem ratio could be used to determine various growth stages of mango nursery trees, and there was a negative correlation between cortex ratio and plant growth vigor.

On the other hand, rough lemon (*Citrus jambhiri* Lush.) which is known as a vigorous citrus rootstock, has a lower rate of stem phloem tissue, compared to weak growing *Poncirus trifoliata* (L.) Raf. rootstock [Saeed et al. 2010]. Decreasing of hydraulic conductivity in consequence of decreasing xylem diameters, might be the reason of the reducing growth vigor of varieties that grafted onto the dwarfing rootstocks of cherry and peach [Végyvári et al. 2008, Tombesi et al. 2010]. And, reduction in root water potential decreased the stomatal conductance and photosynthesis rate respectively [Gonçalves et al. 2007]. The ratio of xylem area to total cross-section area in a plant stem, affected the water potential and uptake of the nutrients dissolved in plant roots. So, the plant nutrient uptake is more high in vigorously growing plants. Moreover, plants with large xylem area grew more vigorous was determined [Simons 1987, Hajagos and Végyvári 2013, Rashedy et al. 2014]. These anatomical features play an important role for the root and stem hydraulic conductivity as proportioned with a total of vessel diameters [Tyree and Ewers 1991, Sellin et al. 2008, Végyvári et al. 2008, Tombesi et al. 2010, Zach et al. 2010]. It was observed that in vigorously growing plants, as the size of xylem vessel diameter decreased, their density increased and the rate of cortex/xylem area in the total section decreased. In fact, in vigorous cherry trees, the vessel diameter of entire plant organs were markedly larger in comparison with the dwarf growing ones. Moreover, an increment in xylem vessel frequency was also predicted in dwarf cherries [Gonçalves et al. 2007, Zorić et al. 2012]. And the trees grafted onto the dwarfing rootstocks had significantly lower xylem/phloem thickness ratio than grafted onto the invigorating once [Gonçalves et al. 2007]. In many tree species, xylem features of root and stem have been significantly affected the growth potential of the rootstock [Castro-Diez et al. 1998, Trifilo et al. 2007, Zorić et al. 2012]. In studies on peach trees, water potential and transport of rootstocks were highly effective on shoot growth, and the main reason of this case

was in relation with the structure of vascular tissues, was suggested [Basile et al. 2003, Solari et al. 2006]. Végyvári et al. [2008], examined the vascular structures of some cherry rootstocks and they notified that, Gisela 5 rootstock had a more narrow xylem surface, while the xylem surface area was more wide in *P. mahaleb* and *P. avium*. Moreover, when compared with the other rootstocks, Gisela 5 had the shorter tracheids as predicted. They also stated that water transport could be limited in this rootstock, so the resistance to summer heats might be lesser. It was observed that vertical transport of the plant nutrients was more limited in dwarfing rootstocks of apple and peach, compared to semi-dwarfing and vigorous rootstocks [Atkinson et al. 2003, Basile et al. 2003]. Tombesi et al. [2010], examined the relationship between xylem vessels and hydraulic transport. In this work, the effects of diameter and density measurements in xylem tissues of Nemaguard, P 30-135 and K 146-43 rootstocks on growth vigour were determined. They predicted that, xylem areas were ranked from large to small as Nemaguard, P 30-135 and K 146-43. Despite the wider xylem area, vessel density was lesser and the hydraulic conductance was higher in Nemaguard rootstock compared to the others. This histological feature could be used to predict the growth vigour among the mentioned rootstocks was concluded. Turkey is an important gene center of some plum species together with a very rich diversity of plum genotypes. Propagation has been mostly conducted with seedling rootstocks and the seeds have been collected from wild [Bolat et al. 2017]. For this reason, the rootstock features of selected genotypes need to be determined for breeding strategies.

In this study, to predict the plant growth vigor in plum rootstock breeding, a novel method that would accelerate the rootstock selection by using the features of vascular elements besides the growth vigor as pre-selection criteria was aimed.

MATERIAL AND METHOD

Material. The material of this study consisted upon 12 wild *P. cerasifera* Ehrh. plum genotypes placed on the mother block of Protection of Plant Genetic Resources of Aegean Agricultural Research Institute in İzmir/Turkey. Registered rootstocks like Gediz EK

A 56-2, Myrobolan 29C and Pixy, having different growth vigor features, which were formerly predicted in different studies were used as control.

Method. Nursery tree production from selected genotypes (with the exception of clonal rootstocks) was conducted by seeds. Seeds were collected in June–August 2012 and they were stratified at +4°C during the autumn period. And they were sown in 2 L pots in January 2013 and they were grown until January 2014. They were transplanted to the experimental plot in March 2014. The morphological parameter, tree height was measured at the beginning and the end of the vegetation periods of two consecutive years. Measurements were conducted in March 2015 and 2016 respectively. A completely randomized design was used for the measurements of three replicates of each genotype. For histological studies, samples were taken from the internodes of the median portions of one-year-old shoots (4–6 mm in diameter), sprouted from the lateral branches of two-years-old nursery trees. Microwave oven irradiation technique modified from Schichnes et al. [2001] was used in histological studies. Cross and longitudinal sections, in 25 µm thickness were taken with a rotary microtome. Samples were staining with aniline blue. Data were collected by measuring the tracheary elements from four different watching areas of three different sections in each replication, which were randomly selected. In measurements, Argenit Kameram software (<http://argenit.com.tr>) was used to examine the sections, which was working with the light-contrast system, through use of Delphi based, high algorithmic processing technique (Fig. 1A–C). Samples were examined and photographed under a Carl Zeiss Axiscop 2 light microscope and the measurements were done at 4× magnification.

The examined histological parameters are as follows:

1. Xylem vessel area (µm²): xylem vessel (trachea and tracheids) area ratio to the number of xylem vessels in measured cross-section area.
2. Xylem vessel area (µm²) = xylem vessel area / xylem vessel number × 100
3. Xylem diameter (µm): diameter length of xylem area in measured cross-section area (Fig. 1B).
4. Xylem area ratio (%): total xylem area ratio to total section area.

5. Xylem area ratio (%) = [total xylem area (µm²) / total section area (µm²)] × 100.

6. Cortex area ratio (%): total cortex area ratio to total section area.

7. Cortex area ratio (%) = [total cortex area (µm²) / total cross section area (µm²)] × 100.

8. Cortex/xylem area ratio (%): total cortex area ratio to total xylem area.

9. Cortex/xylem area ratio (%) = [total cortex area (µm²) / total xylem area (µm²)] × 100.

10. Xylem vessel length (µm): mean length of a xylem vessel measured in longitudinal section (Fig. 1C).

11. Total xylem vessel number: total number of xylem vessels measured in cross-section.

12. Total xylem vessel area (mm²): total xylem vessel area measured in cross-section area.

To predict the growth vigor of the plum genotypes, a completely randomized simple factorial design with three replications (1 plant per each) was used. Measurements were done on three shoot samples taken from each plant. The xylem elements (trachea and tracheids) were measured from four different monitoring area of three different sections, randomly selected from each replication. Correlations between parameters were determined by Pearson's correlation test. Statistically significant ($p \leq 0.05$) correlations were given.

RESULTS AND DISCUSSION

Results showed that a negative correlation between tree height and xylem vessel area ($r = -0.468$, $p \leq 0.05$), and as the tree height decreased, xylem vessel area increased (Tab. 1, Fig. 2A). The mean tree height of Pixy, which is known as a dwarfing rootstock, was in the last statistical group, but at the same time it was in the first group in accordance with xylem vessel area. On the other hand, Gediz A EK 56-2 was in the first group related with the mean plant height, but it was in the last group, when the mean xylem vessel areas were measured. In cherry and peach, the number and diameters of xylem vessels in the roots of rootstocks have a strong influence on growth vigor of scion [Gonçalves et al. 2007, Tombesi et al. 2010]. Generally, the wider vascular strands together with the lesser density of vascular elements, were found to be in relation with the

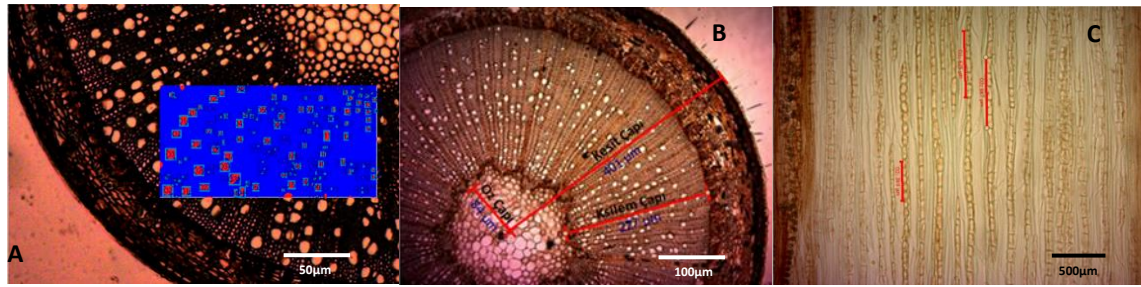


Fig. 1. Xylem area measurements in cross-section (A). Diameter measurements in cross-section (B). Xylem vessel length measurements in longitudinal section area (C)

Table 1. Correlation coefficients between investigated parameters

Correlation Parameters	Tree height (cm)	Xylem vessel area (μm ²)	Xylem diameter (μm)	Xylem vessel length (μm)	Xylem area ratio (%)	Cortex area ratio (%)	Cortex/xylem area ratio	Total xylem vessel number	Total xylem vessel area (mm ²)
Tree height (cm)	1,000	-0,468*	0,577*	-0,477*	0,425*	-0,445*	-0,427*	0,597	0,678*
Xylem vessel area (μm ²)	-0,468*	1,000	-0,388*	0,426*	-0,266*	0,243*	0,286*	-0,645	-0,462*
Xylem diameter (μm)	0,577*	-0,388*	1,000	-0,441*	0,656*	-0,614*	-0,561*	0,692	0,874*
Xylem vessel length (μm)	-0,477*	0,426*	-0,441*	1,000	-0,411*	0,379*	0,411*	-0,392	-0,426*
Xylem area ratio (%)	0,425*	-0,266*	0,656*	-0,411*	1,000	-0,968*	-0,957*	0,447	0,560*
Cortex area ratio (%)	-0,445*	0,243*	-0,614*	0,379*	-0,968*	1,000	0,957*	-0,422	-0,530*
Cortex/xylem area ratio	-0,427*	0,286*	-0,561*	0,411*	-0,957*	0,957*	1,000	-0,387	-0,485*
Total xylem vessel number	0,597*	-0,645*	0,692*	-0,392*	0,447*	-0,422*	-0,387*	1,000	0,872*
Total xylem vessel area (mm ²)	0,678*	-0,462*	0,874*	-0,426*	0,560*	-0,530*	-0,485*	0,872*	1,000

* – correlations found to be statistically significant ($p < 0,05$)

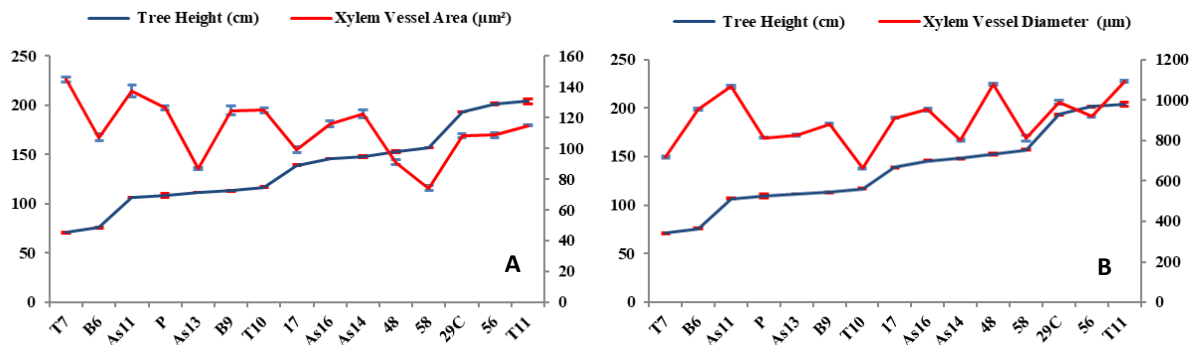


Fig. 2. Relationship between tree height and xylem vessel area (A). Relationship between tree height and xylem vessel diameter (B). (P: Pixy, 29C: Myrobolan 29-C, 56: Gediz EK A 56-2). Vertical bars indicated standard error of mean

growth vigor in fruit and forest species [Castro-Diez et al. 1998, Zach et al. 2010, Chen et al. 2015]. Obtained results were in parallel with the results of the former works.

There was a positive correlation between tree height and xylem vessel diameter ($r = 0.577, p \leq 0.05$), and as the xylem vessel diameter increased, the tree height increased (Fig. 2B). It was observed that, Pixy was in the last statistical group in terms of the mean tree height and mean xylem diameter. As for the Gediz A EK 56-2 rootstock, it was in the first group in accordance with the mean tree height and xylem diameter. In fact, the ratio of xylem diameters in different sizes was accepted as a useful indicator to predict the dwarfing potential of mango cultivars [Rashedy et al. 2014]. In fact, vessel diameter of stems were significantly higher in invigorating cherry rootstocks and pear phenotypes was reported [Gonçalves et al. 2007, Zorić et al. 2012, Chen 2015].

A positive correlation between tree height and xylem area ratio was found ($r = 0.425, p \leq 0.05$), and tree height increased in parallel with the increase in xylem area ratio (Tab. 1, Fig. 3A). Pixy rootstock was in the last statistical group as opposed to Gediz A EK 56-2 which was in the first group, in relation with both parameters mentioned above. As a matter of fact, in studies on growth vigor of olive cultivars and rootstocks, positive correlations were also predicted between plant growth vigor and xylem area ratios [Trifilo et al. 2007].

A negative correlation between tree height and cortex area ratio was observed ($r = -0.445, p \leq 0.05$ – Tab. 1, Fig. 3B). In Pixy rootstock mean tree height was in the last statistical group, while the cortex area ratio was in the first group. As for the Gediz A EK 56-2, it was in the first group in accordance with plant height, while it was in the last group related with the cortex area ratio as opposed to Pixy. In a similar study, Majumdar et al. [1972] reported that, the cortex and xylem rates could be used to predict the growth stages in mango nursery trees. Moreover, they predicted a negative correlation between cortex percentage and plant growth vigor. In pear phenotypes, cortex area ratio was found significantly higher in dwarf type while the xylem area ratio was higher in standard one [Chen et al. 2015]. On the other hand, one of the vigorous citrus rootstocks, rough lemon had the lower stem phlo-

em percentage compared to weak rootstocks like trifoliolate orange was observed [Saeed et al. 2010]. From this point of view, the ratio of different tissues in total stem cross-section area seems to be reliable indicators of plant growth vigor.

A negative correlation was observed between tree height and cortex/xylem area ratio ($r = -0.427, p \leq 0.05$). As the cortex/xylem area ratio increased, tree height decreased (Tab. 1, Fig. 4A). In Pixy rootstock, mean tree height was in the last statistical group, while it was in the first in terms of cortex/xylem area ratio. Gediz A EK 56-2 was in the first group in relation with the mean plant height. But it was in the last group when the mean cortex/xylem area ratio was considered. Kurian and Iyer [1992], compared the xylem/phloem area rates of 24 mango cultivars having different growth vigor, and they concluded that there was a negative correlation in accordance with growth vigor and xylem/phloem area ratio. Similar results were formerly observed in dwarfing apple rootstocks [Lockard 1976].

There was a negative correlation between tree height and xylem vessel length, and as the vessel length increased, tree height decreased ($r = -0.477, p \leq 0.05$) – Table 1, Fig. 4B. In terms of mean plant height, Pixy rootstock was in the last statistical group while it was in the first group, when the mean xylem length figures were examined. As for the Gediz A EK 56-2, it was in the first group in terms of mean plant height, but in the last group in relation with the mean xylem vessel length. This result seemed somewhat conflicting with the findings that pointed out a positive relationship between vessel length and growth vigor in cherry rootstocks [Zorić et al. 2012].

It was observed that, there was a positive correlation between the tree height and total xylem vessel number ($r = 0.597, p \leq 0.05$). As the number of vessels increased, the tree height also increased (Tab. 1, Fig. 5A). The mean tree height of Pixy rootstock was in the last statistical group, while Gediz A EK 56-2 was in the first group. Despite the non-significant differences were observed between genotypes in relation with the total xylem vessel number, Gediz A EK 56-2 had the higher number of vessels than Pixy, in two consecutive years. Similar results were also observed in avocado (*Persea americana* Mill.) rootstocks, and a positive correlation found between growth vigor and

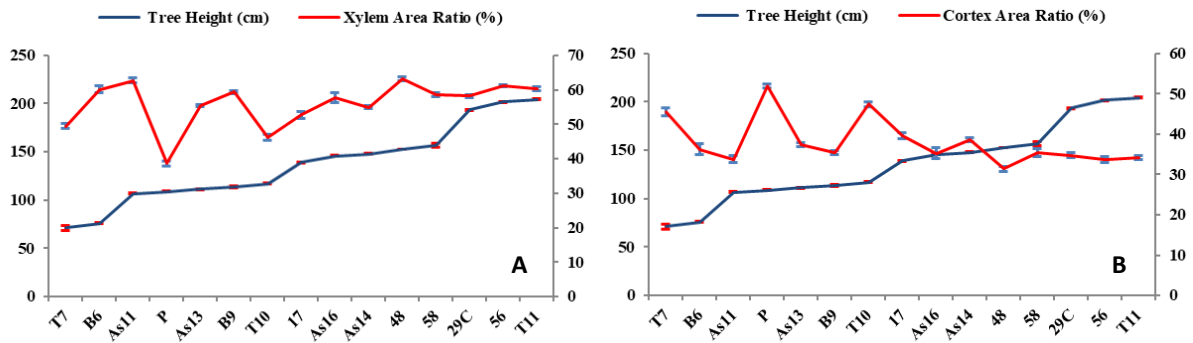


Fig. 3. Relationship between tree height and xylem area ratio (A). Relationship between tree height and cortex area ratio (B). (P: Pixy, 29C: Myrobolan 29-C, 56: Gediz EK A 56-2). Vertical bars indicated standard error of mean

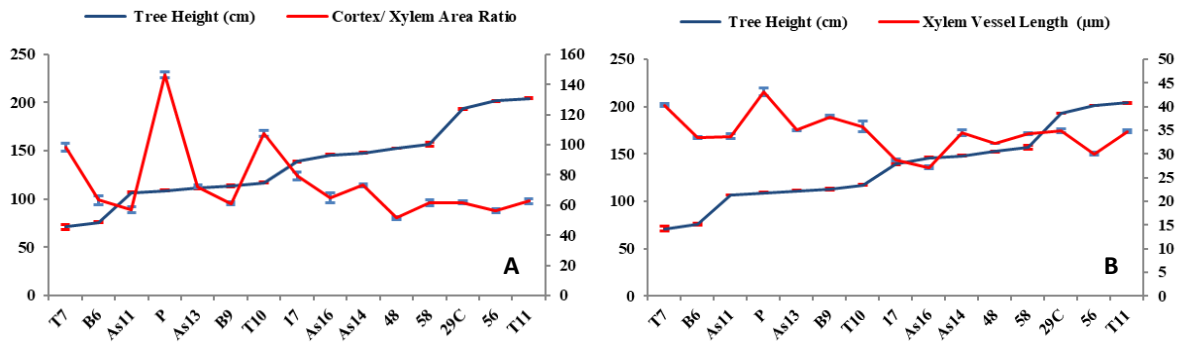


Fig. 4. Relationship between tree height and cortex/xylem area ratio (A). Relationship between tree height and xylem vessel length (B). (P: Pixy, 29C: Myrobolan 29-C, 56: Gediz EK A 56-2). Vertical bars indicated standard error of mean

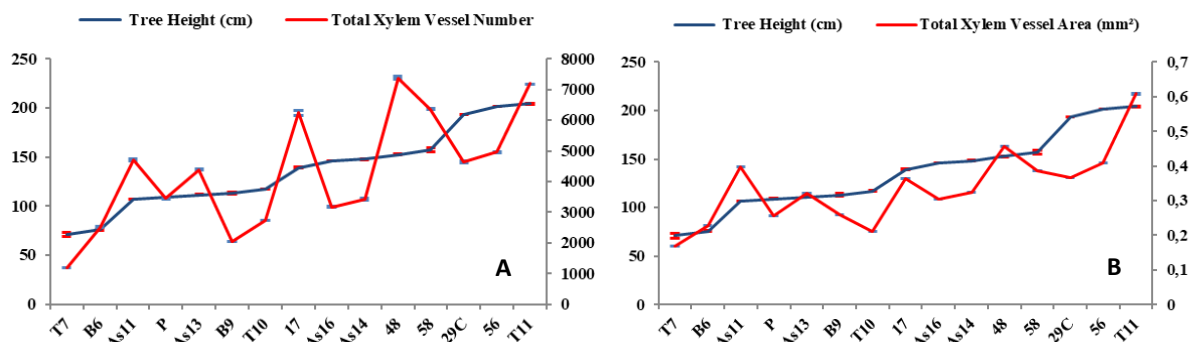


Fig. 5. Relationship between tree height and total xylem vessel number (A). Relationship between tree height and total xylem vessel area (B). (P: Pixy, 29C: Myrobolan 29-C, 56: Gediz EK A 56-2). Vertical bars indicated standard error of mean

xylem vessel number [Fassio et al., 2009]. Moreover, stem vessel frequency proposed as a reliable parameter in vigor prediction of cherry rootstocks studied [Zorić et al. 2012]. For this reason, rapid and precise calculation of vessel numbers by a proper software could be a prominent method in pre-selection studies was thought.

A positive correlation was observed between the tree height and the total xylem vessel area ($r = 0.687$, $p \leq 0.05$ – Tab. 1, Fig. 5B). Tree height increased in parallel with the increase in vessel area. Pixy rootstock was in the last statistical group, while Gediz A EK 56-2 was in the first group, in relation with the mean plant height. Similar results were also reported in different cherry rootstocks compared with stem average vessel area [Hajagos and Végvári 2013].

Water uptake has been started from roots by turgor pressure. Presence of the lower xylem vessel area together with the higher xylem vessel number and xylem area, gives rise to the higher turgor pressure. Thus, it may lead to the higher sap-water flow and an increase in growth vigor respectively. In this study, size and density of xylem vessels influenced the plant growth when the all parameters were considered. Knowing the histological features of rootstocks is of importance to understand that how rootstock affects the growth of scion. Particularly, water uptake and transport via xylem are necessary to substitute of water losses by transpiration, to protect the plant from desiccation and to allow the continuity of photosynthesis [Kramer and Boyer 1995].

CONCLUSION

In this study, the software used for measurements was firstly introduced and used in a plant anatomical study focused on rootstocks selection. It was quite useful and practical for quick and accurate measurement of the vascular structures for early selection in plum and probably the other stone fruit rootstocks were exposed. Significant correlations were found between the anatomy of vascular structures and plant growth vigor of some plum genotypes could be potentially used as rootstock in this study. In terms of xylem vessel area, genotypes which take part together with Pixy rootstock in the same statistical group showed less growth vigor than those in the same group with

Gediz A EK 56-2 rootstock. When the cortex/xylem area rate examined, genotypes taking part in the same group together with Gediz A EK 56-2 rootstock showed higher growth vigor. In terms of xylem vessel length, genotypes taking part with Pixy rootstock in the same group showed less growth vigor, compared to the genotypes which are in the same group together with Gediz A EK 56-2 rootstock. When the entire genotypes compared, T7 and B9 genotypes were the most dwarf, while 17 and B6 genotypes were the most vigorous was exposed. In conclusion, further studies should be needed to determine the other cultural features of dwarfing rootstock genotypes in particular.

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