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Histological Indicators of Dwarfism of Some Olive Cultivars

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Abstract: The current study was conducted during 2011 season on eleven olive cultivars namely “Picual”, “Manzanillo”, “Teffahi”, “Aggezi”, “Chemlali”, “Dolce”, “Koroneiki”, “Frantoio”, “Coratina”, “Arbequina” and “Cairo7”. Stem anatomy conducted to examine the relation between internal structure and plant vigours. Measurements on stem cross section showed that vigour cultivars had higher xylem and low phloem percentage and high number of the large vessels. The stem anatomy indicated that Cairo7 cv. had the highest dwarfing potential.

Key words: Olive · Anatomy · Xylem · Phloem · Dwarfism

INTRODUCTION

Olive (*Olea europaea* L.) is one of the most ancient cultured plants. It was originated in the eastern side of the Mediterranean basin and spread to other countries. Despite the large number of cultivars selected from the beginning of olive domestication, the profound changes in olive growing in recent years have revealed the significant disadvantages of most of traditional cultivars for new olive orchards. The availability of small size trees is the main condition for the intensification of planting systems, along with using machines of high operative efficiency. Recently, super high density olive growing systems had been set up with the use of specific low-vigor and compact canopy cultivars [1].

Several hypotheses had been proposed to explain the effect of rootstock on scion growth. Some authors suggested that reduced growth induced by dwarfing rootstock may be related to anatomical features of the rootstock. Since, the difference in rootstocks genotype vigor was reflected in roots and young shoots anatomy, it appeared that it may be possible to screen for size-control genotype in seedling population by quick examination of stem anatomy of seedling shoots [2].

The goal of the current research was to study the histological characteristics of some olive cultivars which may be associated with their growth behavior.

MATERIALS AND METHODS

The present study was carried out during 2011 season at the Nursery and Laboratory of Pomology Department, Faculty of Agriculture, Cairo University. Eleven olive cultivars with markedly different growth behavior were selected; self-rooted cutting of olive cultivars namely “Picual”, “Manzanillo”, “Teffahi”, “Aggezi”, “Chemlali”, “Dolce”, “Koroneiki”, “Frantoio”, “Coratina”, “Arbequina” and “Cairo7”. One year old plants grown in plastic bags filled with mixture of sand and compost were grown in the greenhouse and received the recommended agriculture practices.

Stem samples of the studied cultivars were collected and immediately fixed in F.A.A., dehydration of the samples was performed in increasing concentration of ethyl and butyl alcohol and then the samples were infiltrated with paraffin wax. The samples were cross sectioned by rotary microtome and stained with Safranin (Staining in red the lignified cell walls) and Light green (staining in blue green cellulose walls) and examined under light microscope [3, 4]. Images were captured by light microscope supplement with camera (Panasonic WV-CP 220, Japan).

The obtained images were subjected to analysis by image analysis software (Digimizer software package) and the following parameters were calculated: xylem area (mm^2), xylem percentage, phloem area (mm^2), phloem percentage, xylem /phloem ratio, number of vessel elements per cross section area, the mean vessel element area (μm^2), frequency distribution of xylem vessel size per cross section, xylem vessel density (number of vessel per unit area) and total conduit area (vulnerability index).

The obtained data were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran [5] using MSTAT-C statistical package software [6].

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Means of the treatments were compared by Least Significant Difference (L.S.D.) according to Duncan [7] at significance level of 0.05.

RESULTS AND DISCUSSION

The stem anatomical features of the eleven olive cultivars under investigation as seen in transverse sections are shown in Figure (1). Generally, it is realized that olive stem structure showed typically dicotyledons herbaceous stem structure that characterized by Eustele with remarkable thick cortex and pith. The histological measurements of the studied cross sections showed relatively advanced degree of secondary growth. As well developed periderm exhibited comparable thickening among cultivars. The most thickened one was associated with "Koroneiki" followed by "Dolce" then "Chemlali". While, the other studied olive cultivars showed relatively same periderm thickness. Moreover, it is evident, that the cortex showed complete ring of parenchyma that surround the stele. This ring in broad showed parenchyma cells with good constancy that may have numerous iodoplasts cells with numerous quantities of druses crystals.

The relation between each component of the stem cross section and the dwarfing potential were discussed under the following sections.

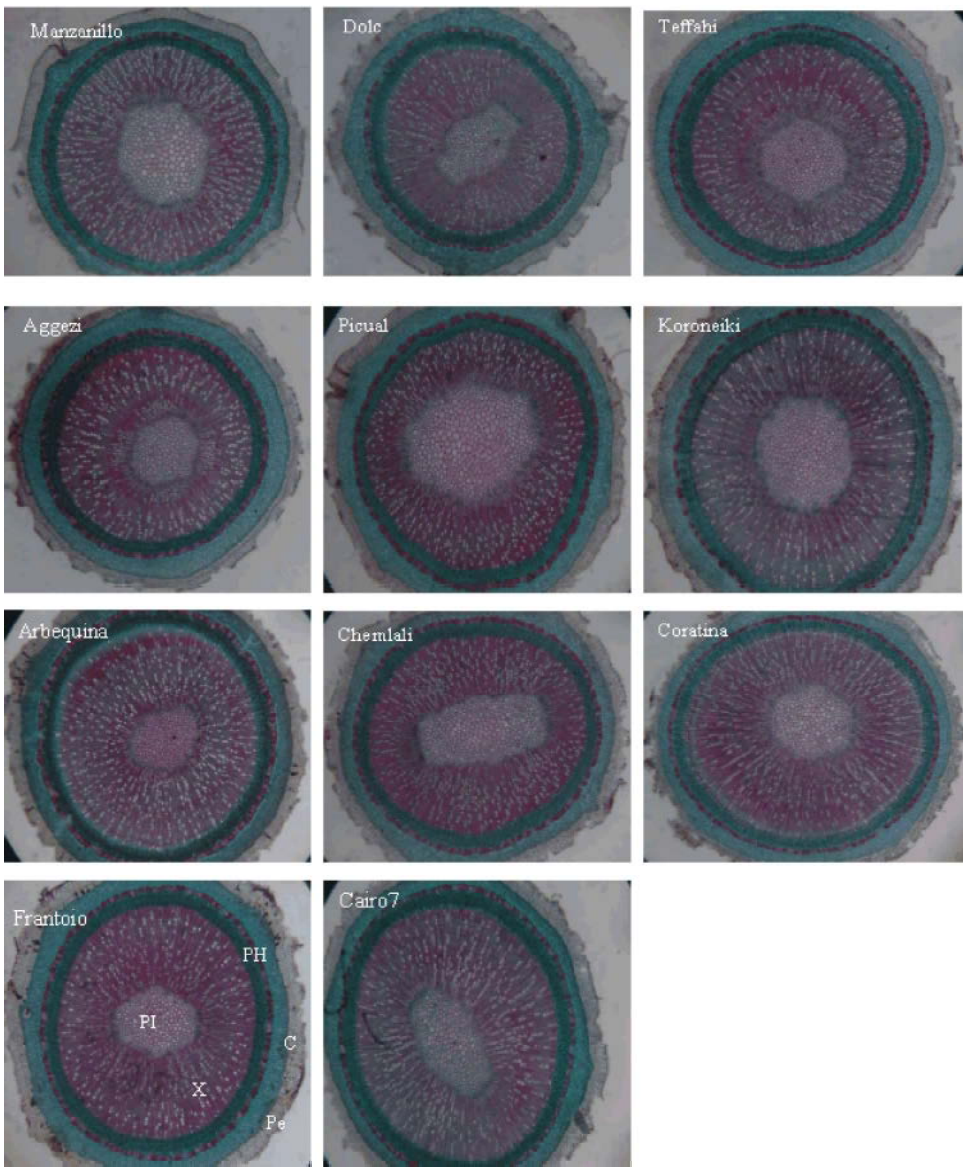

Fig. 1: Stem cross section of olive cultivars; Pe, (periderm), C (Cortex), PH (Phloem), X (Xylem), PI (Pith).

Table 1: Xylem area and percentage of the studied olive cultivars

Cultivars	xylem area (mm^2)	xylem percentage	angle of xylem percentage
Dolce	2.517 e	39.528	38.96 g
Manzanillo	3.136 cd	48.750	44.28 b
Aggezi	2.466 e	41.251	39.96 fg
Teffahi	2.991 d	43.301	41.15 ef
Picual	4.115 b	46.414	42.94 b-d
Koroneiki	4.293 b	47.935	43.82 bc
Arbequina	4.255 b	45.143	42.21 de
Chemlali	4.310 b	48.549	44.17 b
Coratina	4.858 a	52.231	46.28 a
Cairo 7	3.956 b	45.985	42.70 cd
Frantoio	3.451 c	42.285	40.56 f

Means within each column with the same letter (s) are not significantly different at $P < 0.05$

Table 2: Phloem area, percentage and xylem/ phloem ratio of the studied olive cultivars

Cultivars	phloem area (mm^2)	phloem percentage	angle of phloem percentage	xylem/ phloem ratio
Dolce	0.830 cd	13.038	21.17 bc	3.039
Manzanillo	0.751 d	11.650	19.69 cd	4.351
Aggezi	0.753 d	12.593	20.79 cd	3.286
Teffahi	1.193 ab	17.264	24.55 a	2.538
Picual	1.007 bc	11.367	19.70 d	4.084
Koroneiki	1.114 b	12.452	20.66 cd	3.865
Arbequina	1.196 ab	12.681	20.86 b-d	3.582
Chemlali	1.016 bc	11.453	19.78 cd	4.255
Coratina	1.332 a	14.336	22.25 b	3.651
Cairo 7	1.066 b	12.387	20.61 cd	3.760
Frantoio	1.033 b	12.680	20.86 b-d	3.342

Means within each column with the same letter (s) are not significantly different at $P < 0.05$

Xylem Area and Xylem Percentage: The area of xylem tissue in the studied olive cultivars as shown in Table (1) indicate that Coratina had the highest xylem area followed by “Koroneiki” and “Chemlali”. While “Aggezi” and “Dolce” recorded the lowest values. The area of the xylem determines the potential of water and soluble transport through the stem and thereby had a direct effect on the plant growth vigor.

According to Trifilo *et al*. [4] xylem cross section area was 3.53 mm in the vigor “Leccino Minerva” olive clone, while it was only 2.57 mm^2 in the “Leccino dwarf”. The xylem percentage in the studied cultivars as shown in Table (1) reveal that “Coratina” had the highest xylem percentage followed by “Manzanillo”, “Chemlali” and “Koroneiki”, while “Dolce” and “Aggezi” had the lowest percentage. It is obvious that there was a positive relation between growth vigor and xylem percentage. According to Tombesi *et al*. [2] the anatomical analysis of xylem could be a useful means of predicating the vigor control capacity of selected rootstocks genotype.

Phloem Area and Phloem Percentage: As shown in Table (2) the phloem area was higher in “Coratina” and “Teffahi” compared with the other cultivars, while “Aggezi” and “Manzanillo” had the lowest areas. Also, “Teffahi” had the highest phloem % compared with the other cultivars, the phloem percentage was similar in the other cultivars, “Picual” and “Manzanillo” had the lowest percentage.

Phloem thickness is strongly related to olive tree growth. The phloem carries the sugars made in leaves downward to the other trunk parts and the root. The outer bark or periderm reduces water loss and protects the tree from mechanical, heat and diseases damages.

Majumdar *et al.* [8] found that the proportion of bark and xylem can be used to classify mango in various vigor classes in the nursery stage and they found a negative correlation between bark percentage in roots and plant vigor. Also the vigorous rootstock Rough Lemon possessed lower proportions of phloem in the stem when compared with the less vigorous rootstock such *Poncirus trifoliate* [9].

Table 3: Average vessel area, number, density and total conduit area in stem of the studied olive cultivars

Cultivars	Vessel area (mm*10^3)	Vessel number	Total conduit area
Dolce	0.2490 a	737 c	0.127 e
Manzanillo	0.4633 a	753 a	0.367 a
Aggezi	0.4639 a	710 d	0.214 c-e
Teffahi	0.3755 a	646 f	0.243 b-d
Picual	0.4068 a	529 j	0.278 a-d
Koroneiki	0.4750 a	532 j	0.324 ab
Arbequina	0.4112 a	697 e	0.336 ab
Chemlali	0.3434 a	747 b	0.329 ab
Coratina	0.4032 a	569 g	0.369 a
Cairo 7	0.4324 a	561 h	0.292 a-c
Frantoio	0.3607 a	549 i	0.195 de

Means within each column with the same letter (s) are not significantly different at $P < 0.05$

Xylem /phloem Ratio: Data in Table (2) show that "Manzanillo" recorded the highest xylem to phloem ratio followed by "Chemlali" and "Picual", while "Teffahi" had the lowest xylem to phloem ratio. An intermediate xylem/phloem ratio was scored with "Koroneiki", "Aggezi" and "Cairo7" that reflects on their transitional vigor growth and redirects their dwarf habitat.

The xylem / phloem ratio is one of the main anatomical characteristics which are frequently used in classification of rootstocks. Kuriana and Iyer [10] in studies conducted on 24 mango cultivars of varying vigor; they indicated that low vigor of trees was associated with higher phloem to xylem ratio in shoots. The slower growth reduced height for dwarfing rootstocks like "Fly Dragon" was attributed to high bark /wood ratio in the stem [9]. Similar observations were recorded in the dwarfing apple and peach rootstocks [11-13].

Number of Vessel Elements per Xylem of Stem Cross Section: The number of xylem vessel varied between the studied cultivars, data in Table (3) show that "Manzanillo" and "Chemlali" had the highest vessel number while "Picual" and "Koroneiki" had the lowest number. According to Goncalves *et al.* [14] there was a lower xylem conduit in trees grafted on dwarfing rootstocks than trees on the vigor rootstocks.

Also plant height had a negative correlation with the number of vessel elements in the xylem of stem, the dwarf "Fly Dragon" had the highest vessel frequency (140.6 vessel/ mm), while the vigor "Rough Lemon" rootstock had the lowest vessel frequency (48.7 vessel/ mm) [9].

According Raimondo *et al.* [15] the number of conduits was higher in the dwarf olive rootstock "Leccino dwarf" (233 conduits/ mm), while the number was only 209 conduits/mm in the vigour olive rootstock "Leccino Minerva".

The Mean Vessel Element Area: The average area of the vessel unit of the studied cultivars is shown in Table (3), Koroneiki and Aggezi had the highest average conduit while Dolce had the lowest value.

The mean xylem vessel diameter is a genetically trait associated with rootstocks vigor that was expressed in the roots and stems of peach rootstocks genotypes [16]. The anatomical measurement showed that "Leccino Dwarf" had narrower xylem conduits than "Leccino Manerva" clone [15].

Total Conduit Area (Vulnerability Index): The data presented in Table (3) show that "Coratina" and "Manzanillo" had the highest total conduit area followed by "Arbequina", while "Dolce" had the lowest value.

Data presented in Table (3) indicated that, the studied olive cultivars "Coratina" and "Manzanillo" had the highest average conduit area followed with insignificant differences by "Arbequina", "Chemlali", "Koroneiki" and "Cairo7", while "Dolce" showed the lowest average conduit area. So, this trait could be used as indicator of dwarfism in the studied olive cultivars. This could be explained by the findings of Tirfilo *et al*. [4] who reported that the potential cross section area was greater in the vigor olive clone "Leccino Minervo" compared with the dwarf clone "Leccino dwarf". Moreover, Fassio *et al*. [17] studied the relation between sap flow and xylem anatomy in two avocado rootstocks (D.7 and TC) root anatomy of both rootstocks, showed that, D.7 had 19% higher total vessel area than TC. These results suggested that differences in the efficiency of the root to absorb water through the conductive tissue depend on xylem anatomy.

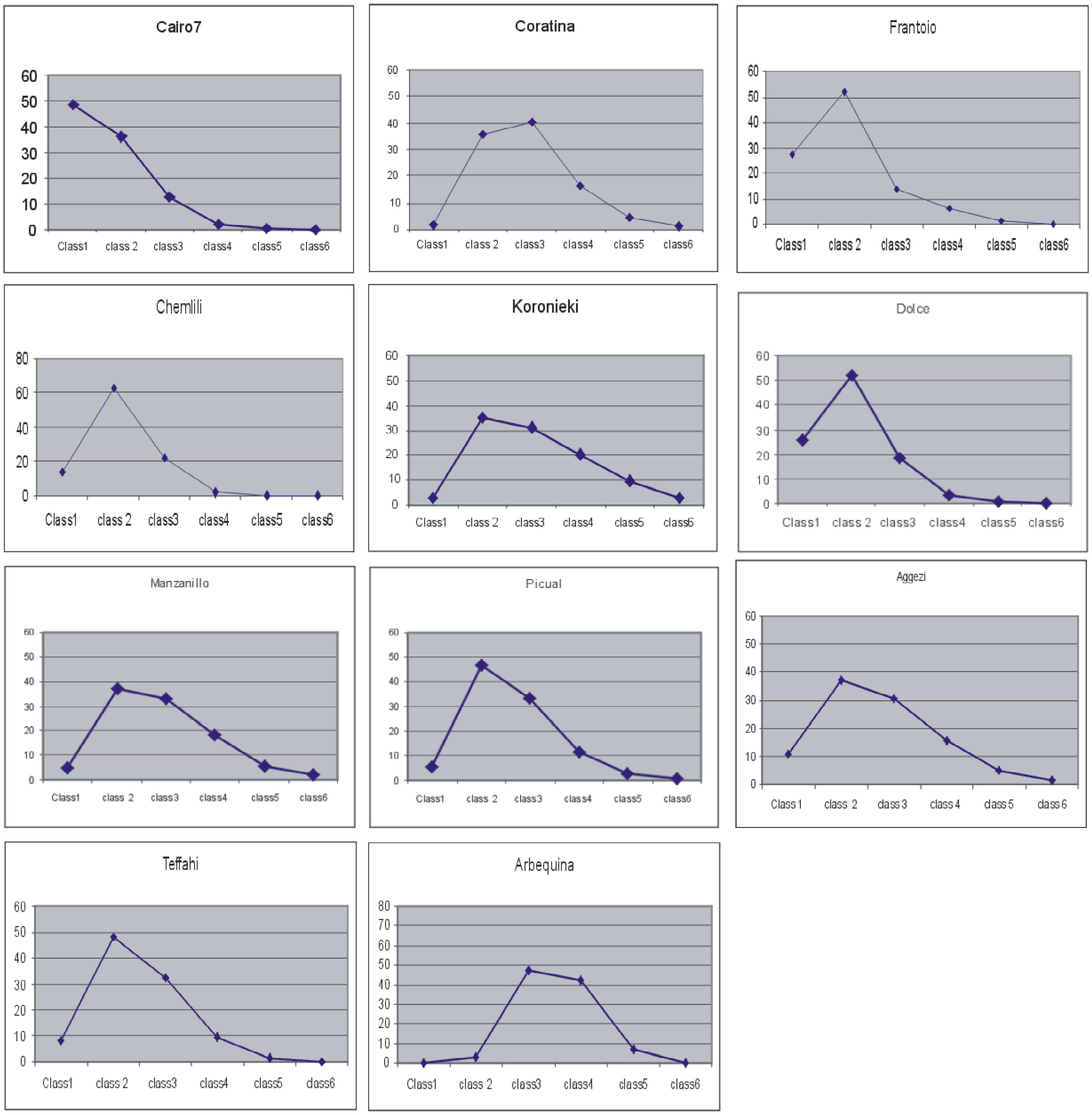

Fig. 2: Percentage of xylem vessels in the different size classes per stem cross section

Vulnerability index showed a significant difference among the studied avocado genotype, "Fuerte" and "Hass" had the highest value whereas the dwarf "Colin V-33" had lower vulnerability index [18].

Percentage of Xylem Vessels in the Different Size Classes per Stem Cross Section: The xylem vessels varied in area of each stem section, the xylem vessels are classified into 6 categories (Fig 2). Class 1 less than 10µm, Class 2 from10 to 15µm, Class 3 from 16 to 20µm, Class 4 from 21 to 25µm, Class 5 from 26 to 30µm, Class 6 more than 30μm^2

The percentage of the vessels in the different categories varied greatly between the studied cultivars, the studied cultivars can be classified into 3 groups according to the pattern of vessel area distribution:

- The first group including "Cairo 7", "Frantoio", "Dolce" and "Chemlali" which are characterized by high percentage of small vessels and lower percentage of large vessels.
- The second group including "Picual", "Teffahi" and "Aggezi" which had medium number of vessels in each category.

- The third group including “Coratina”, “Manzanillo”, “Koroneiki” and “Arbequina” which are characterized by low percentage of small vessels and high percentage of large vessels.

The analysis of xylem vessel of the studied cultivars showed that, “Cairo 7” had the highest percentage of vessels of the low area (48.13% from the first class and 36.33% from the second class) followed by “Frantoio”, “Dolce” and “Chemlali”. While “Coratina” had the lowest number of vessels in the small area (2% from the first class and 35 from the second class) followed by “Arbequina” and “Manzanillo”.

Reversely, “Koroneiki” had the highest percentage of the large vessels (2.44% from the 6^{th} class, 9.21% from the 5^{th} class and 20.11% from the 4^{th} class) compared with the other cultivars followed by “Manzanillo”, “Coratina” and “Arbequina”.

The dwarfing rootstocks had more vessels in the smaller size classes, while the more vigor rootstocks had more vessels in the larger size classes [2]. According to Tirifilo *et al*. [4] more than 65% of the xylem conduits of the “Leccino Manerva” ranged between 15 and 25 µm in diameter whereas only 15% were less than 15µm in diameter. On the other hand, shoots of the “Leccino Dwarf” plants had significantly narrower conduits *i.e.* over 90% of the conduits were less than 25µm and conduits wider than 25µm were about 12%.

In conclusion, it is possible to screen the dwarfing potential of different olive cultivars by examination of the stem anatomy. Phloem and xylem percentage and the percentage of xylem vessels in different size classes appear to be a good indication of the dwarfing potential. Also, Cairo7 appear to be a promising cultivar with a high dwarfing potential.

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