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Stem and leaf anatomical and physiological characteristics of 'Colín V-33' avocado seedlings

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Abstract: The anatomical and physiological structure of the 'Colín V-33' avocado stem and leaf is described from samples from plants obtained from seed in order to identify genotypes and early selection parameters in a rootstock improvement program for avocado. Eighty-nine plants of 12 months of age were used, where a total of 25 anatomical variables of the stem, leaf, and physiological of leaf were evaluated. A cluster analysis was conducted that generated a hierarchical dendrogram that suggested six groups of plants. Furthermore, from the 25 variables, eight were selected as discriminant when performing a canonical discriminant analysis, the variables that most discriminated for the first canonical component were: stem diameter and density of xylem vessels, for the second: thickness of the stem epidermis, temperature of the stem leaf and stomata length, while for the third: thickness of the cambium, transpiration rate, and stomatal conductance. The genotypes showed a great variation between the groups, the characteristics of these indicated that the genotypes of Group 4 showed some that could be related to small or dwarf plants (smaller stem diameter, high density of xylem vessels, a higher rate of transpiration and stomatal conductance). In contrast to the genotypes of Group 3 which presented opposite characteristics in the previous variables, being able to associate with vigorous plants. The anatomical traits of the stem showed to be highly related to the behavior of the avocado plants. Associating genotypes with physiological and anatomical variables in leaf and stem can have great value for the selection of rootstocks at an early stage of development.

1. Introduction

Fruit trees used for the establishment of plantations are formed by a variety/rootstock combination, where the variety provides the productive part and the rootstock provide the root system of the tree (Dolgun *et al.*, 2009). The rootstocks provide a simplified management of the orchard, increase the productivity, ensure the survival of the trees, control the vigor conferring dwarfism and allowing the use of high planting densities and the water balance of the plant (Solari *et al*., 2006), influence the qualitative and nutritional attributes of the fruits (Remorini *et al*., 2008), also influence the scion development, as well as its adaptation to different types of soil, water stress conditions and other climatic conditions (Giorgi *et al*., 2005), as well as salt stress tolerance (Massai *et al*., 2004).

Selection of the rootstock and the cultivar or varieties to be exploited is an economically important decision, since the degree of productivity and quality of production will depend on the variety/rootstock combinations (Pinochet, 2010), these combinations are in function of the degree of affinity in terms of vascular connections between the graft and the rootstock, because vascular regeneration is a complex process that includes the differentiation of xylem and phloem (Aloni *et al*., 2010).

Avocado production in Mexico is based on the use of seed-derived rootstocks which generate large trees that, over time, complicates the agronomic management. For this reason, one of the priorities in avocado research is to find dwarf cultivars that are highly productive, with good fruit quality and resistant to pests and diseases in order to establish high-density plantations and reduce production costs (Sánchez-Colín *et al*., 1992). The use of rootstocks in intensive avocado cultivation is of great importance and rootstocks for this crop have been reported to influence the size of the tree and its productivity (Bergh and Whitsell, 1962). Potentially dwarfing rootstocks such as 'Wilg' and 'Colin V-33' have been evaluated against 'Duke 7', for their ability to limit the growth of 'Hass' (Roe *et al*., 1995).

'Colín V-33' avocado is a Mexican selection that has low vigor and an expanded growth habit (Roe *et al*., 1995), considered as a possible dwarf rootstock since it has been used as an interstock, with reported reduction of 43% of the tree height on 'Fuerte' (Barrientos Priego *et al*., 1987) and 'Hass' (Barrientos-Villaseñor *et al*., 1999). On the other hand, given the genetic variability for the height of the seedlings of this cultivar (Rubí Arriaga, 1988), it makes it a valuable material for the improvement of dwarf avocado rootstocks. In this regard, Barrientos-Priego *et al*. (1992) have found that the cultivar seedlings produce some dwarfing individuals when used as rootstocks.

Efforts have been made mainly with 'Colín V33' for the selection of dwarf rootstocks that meet the needs of the new production systems, where stomata density has been proposed as a possible pre-selection index (Barrientos-Pérez and Sánchez-Colin, 1982; Barrientos-Priego and Sánchez-Colín, 1987).

Other important features to be investigated are the ones that contribute to the hydric status of the plant and its productivity, in which a series of physiological, anatomical and morphological characteristics of the stem can be measured. At the physiological level, gas exchange can be studied (Vilagrosa *et al*., 2010) and to determine the affinity between variety/rootstock combinations, the anatomical structure of xylem can be studied (Sory *et al*., 2010; Leal-Fernández *et al*., 2013).

The anatomical characteristics of the dimensions of vessel elements and the proportions of xylem and phloem, in stems, are important to be able to define the amount of water that can be transported through them, since as the tissues become larger and the presence of smaller diameter of the vessels (higher pressure for water movement), the amount of water transported will be greater, therefore, there will be a better adaptation of the plants to low humidity conditions (Vasconcellos and Castle, 1994; Reyes-Santamaría *et al*., 2002).

Reyes-Santamaría *et al*. (2002) found vessel elements with smaller diameters, for the avocado genotypes that have less vulnerability to drought. The stomatal density (ED) and the thickness of the epidermis are characteristics that may be related to drought tolerance (Baas, 1982), as they are the most exposed anatomical characteristics of the plant, which represent the last link in the transpiration torrent towards the atmosphere (Faust, 1989).

The use of avocado seedling rootstocks of a local type called "Criollo" is very common but rarely studied from their anatomical and physiological characteristics on stem and leaf, variables that can be useful as a preliminary study to understand their possible role when grafted. The objective of this research was to describe the anatomical and physiological structure of the stem and leaf of plants derived from avocado seed of 'Colín V-33', to select individuals at the seedling level with distinctive characteristics.

2. Materials and Methods

The research was carried out in a greenhouse of the Experimental Field of the Chapingo Autonomous

University, in Chapingo, State of Mexico, located at 19° 29’25.7’’ and 98° 52’24.5’’ with an altitude of 2240 meters above sea level.

Plant material

As plant material, 89 avocado seedling plants of ‘Colín V-33’ were used, that were donated by the Germplasm Bank of the Salvador Sánchez Colín Foundation-CICTAMEX, S.C. located in Coatepec Harinas, State of Mexico. The seeds were established in a black bag of caliber 600 of 26 cm x 35 cm with perforations in the first lower third; using soil, perlite and compost as a substrate (3:1:1; v:v:v), watered twice a week, and located in a glass greenhouse with oscillating temperatures between 35 ± 4°C.

Leaf gas exchange variables

To each of the plants the eleventh leaf, fully expanded and healthy, counted from the base towards the apex of the plant was selected as recommended by Barrientos-Priego *et al*. (2003). Once marked, the variables CO_2 assimilation rate (A), transpiration rate (E), leaf temperature, internal CO_2 concentration and, stomatal conductance was evaluated. A punctual measurement was made between 11:00 and 13:30 hours a day with an infrared gas analyzer (model CI-340, CID Bio-Science). This was done during three days of the month of September of the year 2017 and the measurement was taken in the same plant order every day to avoid more variation in the data taken for each plant. The average of the three values obtained was used in the analysis.

The water use efficiency index (WUE) was calculated based on the variables CO_2 assimilation (A) and transpiration rate (T), using the formula WUE = A/T.

Leaf anatomical variables

After the measurements of gas exchange variables, on the same leaf previously marked, an impression of the underside of the middle part of the lamina using silicone for dental impressions (Exactoden) was taken. By applying transparent nail varnish on the (negative) impression, the positive impression was obtained, which was placed on a slide and fixed with a coverslip.

In the positive impressions of each sample, the image area was calculated with an object micrometer, stomatal density (SD) per mm^2 and epidermal cell density (ECD) per mm^2 were determined. These variables were evaluated in five fields [400x, Ayala-Arreola *et al*. (2010)] in a Motic B3 Professional Series microscope, with the adaptation of a Moticam 480 camera with a 16 mm adapter. In addition, 10 stomata were measured in each sample. Stomata counts, epidermal cells and stomata length measurement were performed with the help of ImageJ 1.52a image analyzer.

With all this information the stomatal index (EI) was calculated, which is equal to:

$$EI = [SDE/ (SD + ECD)] \times 100.$$

Stem anatomical variables

Transverse stem samples were obtained from plants of approximately 1 cm each, which were fixed in 96 % ethanol:100 % glacial acetic acid (2:1; v:v) and processed in an automatic tissue exchanger (Tissuematon Fisher) with 2-ethoxyethanol (cellosolve) and xylene, then transfer to paraffin (55°C) staying 72 hours inside a stove. The paraffin pyramid was made according to Sass (1968) and in a rotary microtome (American Optical, model 820), transverse cuts were made with a thickness of 10 µm. The cut sections were stained for 30 min at room temperature in a mixture of equal volumes of 0.1% aqueous solutions of safranin and fast green, then washed in distilled water for 5 minutes and washed in 2 changes of absolute alcohol for 2-3 min (Bryan, 1955). The stained sections then mounted on slides with coverslips by means of Haupt adhesive and 10 % formalin (Sass, 1968).

Fifteen fields were observed in each preparation per replication (three repetitions). For which the 4x, 10x and 40x lenses were used as appropriate, in a Motic B3 Professional Series microscope, with the adaptation of a Moticam 480 camera with a 16 mm adapter and digital images were obtained. The area of the images was calculated with a slide micrometer and then the cell layers of the tissues and the dimensions of xylem vessel elements were measured with the help of the ImageJ 1.52a image analyzer.

The tissues evaluated were epidermis, parenchyma, phloem fibers, phloem, cambium, xylem, and pith. The thickness of each layer and the total diameter of the stem was measured. For the xylem dimensions number of vessel elements per area (vessel density), vessel element area, major axis of vessel element, minor axis of vessel element, cell wall thickness of two contiguous vessel elements, roundness index (RI) of vessel and Feret diameter were obtained.

Statistical analysis

A cluster analysis was performed using Ward’s minimum variance agglomeration method to generate a hierarchical dendrogram (Núñez-Colín and

Escobedo-López, 2011). The resulted dendrogram was divided according to Hotelling's pseudo-statistical t^2 (Johnson, 1998). A canonical discriminant analysis (CDA) with the Mahalanobis distance was performed to determine the most discriminating variables with the greatest importance that describe the groups (Núñez-Colín and Escobedo-López, 2014). All statistical analysis were performed with the SAS V.9.2 statistical package (SAS Institute, 2009).

3. Results and Discussion

In order to explore the homogeneity within each variable evaluated and based on the coefficients of variation (CV), it was determined that the physiological variables of leaf: CO_2 assimilation rate, stomatal conductance and water use efficiency index (WUE), were the characteristics with the greatest variation (Table 1), presenting CV of 60.84%, 57.65%, and 62.5%, respectively. These characteristics being less stable within the plants studied (more heterogeneous). On the contrary, all the anatomical variables of stem and leaf showed lower coefficients of variation that were below 28%, so they could be considered more homogeneous but still show contrasting features (Fig. 1 and 2).

Cluster analysis

Based on Hotelling's t^2 pseudo-statistic (Fig. 3), the grouping by Ward's method showed the identification of six groups of plants (Fig. 4). Group 1 composed of 21 plants, Group 2 by 9, Group 3 with 13, Group 4 with 13, Group 5 with 16 and Group 6 with 17 plants (Table 2).

In the obtained dendrogram a partition of six groups was used approximately at a cut-off distance R^2 semi-partial close to 0.050 (Fig. 3). The greater distance corresponds to Group 4 confirming that are different from the rest, that was also reinforced with

Table 1 - Mean values of some leaf and stem anatomic variables and leaf gas exchange variables of 'Colín V-33' avocado seedling plants. The highest values are presented in bold for the coefficient of variability

Variables	Maximum	Mean	Minimum	CV (%)
Epidermis (µm)	1.72	1.12	0.74	18.43
Parenchyma (µm)	36.00	19.79	12.48	21.66
Phloem fibers (µm)	22.96	15.99	9.19	20.36
Phloem (µm)	66.46	39.72	21.46	22.56
Cambium (µm)	10.69	7.14	4.24	21.01
Xylem (µm)	115.22	76.05	28.40	25.42
Pith (µm)	98.49	60.02	19.41	27.20
Stem Ø (µm)	197.78	152.08	107.85	14.36
Vessel density by area (vessels/µm^2)	6.13	3.11	1.73	25.20
Vessel area (µm^2)	2688.49	1868.91	1096.34	16.99
Mayor axis of vessel (µm)	40.59	29.40	20.27	14.68
Minor axil of vessel (µm)	22.80	17.63	12.05	15.04
Wall thickness of two cells (µm)	3.57	2.80	2.19	11.06
Roundness index	0.59	0.35	0.18	24.03
Feret Ø	87.80	49.53	30.63	18.48
Leaf temperature (°C)	28.53	26.52	23.23	4.47
CO_2 assimilation rate (umol·m^{-2}·s^{-1})	3.97	1.28	-0.06	60.84
Transpiration rate (mmol·m^{-2}·s^{-1})	2.00	0.89	0.32	45.69
Stomatal conductance (mmol·m^{-2}·s^{-1})	105.18	33.04	9.33	57.65
Internal CO_2 concentration (ppm)	352.87	232.58	67.33	20.53
WUE	4.69	1.56	-0.19	62.50
Stomatal density (stomata/mm^2)	317.65	190.66	105.88	19.80
Epidermis cell density (cells/mm^2)	1267.65	818.88	582.35	17.56
Stomatal index (%)	25.16	19.03	11.78	13.89
Stoma length (µm)	19.64	15.99	13.11	8.60

WUE= Water use efficiency. CV= Coefficient of variability.

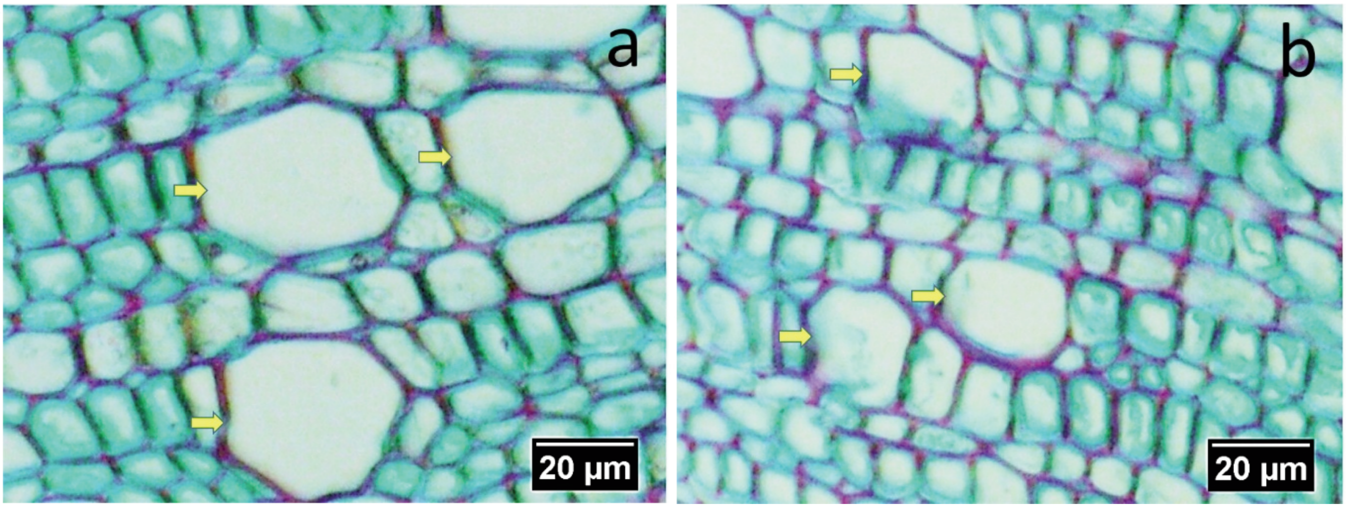

Fig. 1 - Transverse section of xylem tissue of main stem from plant numbered as 110 (a) and 88 (b) of 'Colin V-33' avocado seedlings. Arrows show vessel elements.

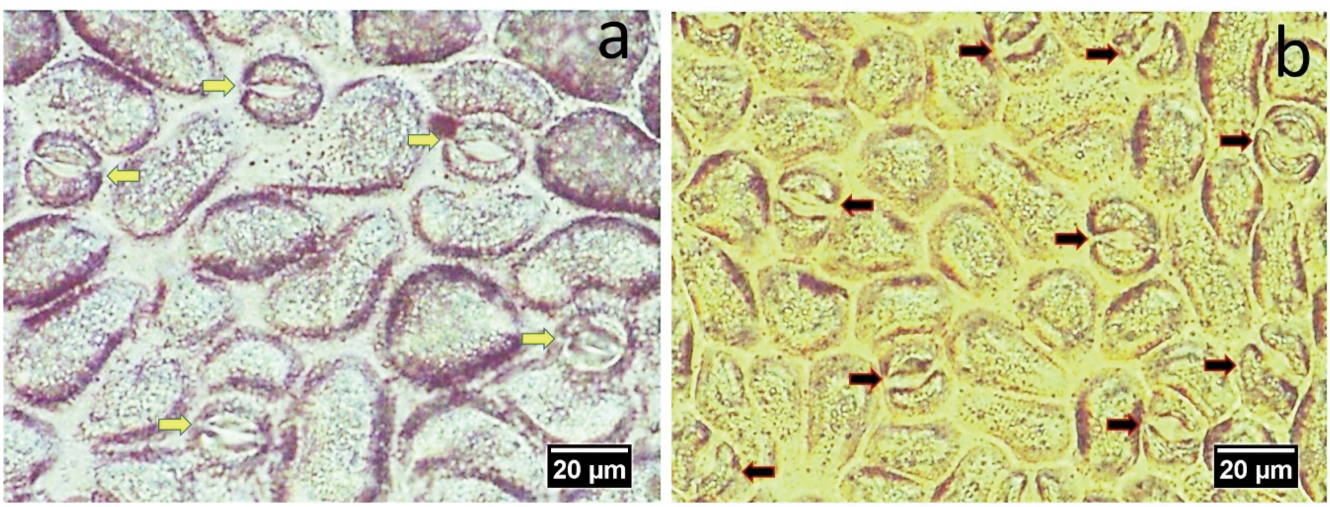

Fig. 2 - Epidermis replicates of leaf lamina from plant numbered as 162 (a) and 96 (b) of 'Colin V-33' avocado seedlings. Arrows show stomata.

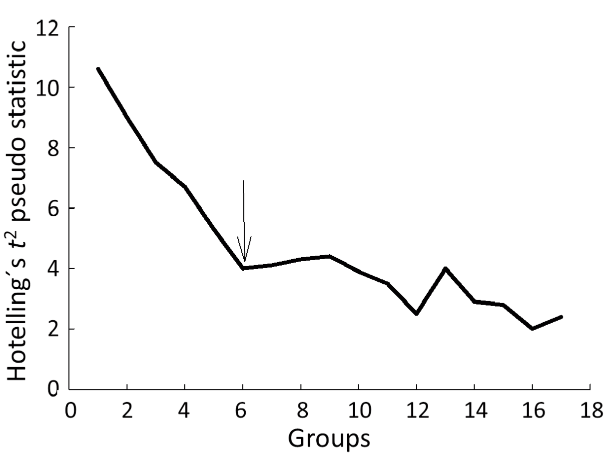

Fig. 3 - Hotelling's pseudo-statistical t^2 and the recommended number of groups as a proof of dendrogram division using physiological and anatomical of leaf and stem variables of 89 'Colin V-33' seedling plants.

plants were developed. Generally, plants with a greater number of relatively small vessel elements are associated with drought-resistant genotypes (Vasconcellos and Castle, 1994; Reyes-Santamaría *et al*., 2002; Núñez-Colín *et al*., 2006).

The CC 2 represented 28.90% of the total variation and was associated with: epidermis thickness, leaf temperature, and stoma length (Table 3 and 4). The anatomical stem variable (epidermis thickness) present in CC 2 is associated with drought resistance (Baas, 1982) and it is possible to relate it as acquired adaptation, to tolerate prevailing environmental conditions of the greenhouse (38°C). On the other hand, the size of stomata is one of the anatomical variables of the leaf that could be sensitive to the change in environmental conditions (Hetherington and

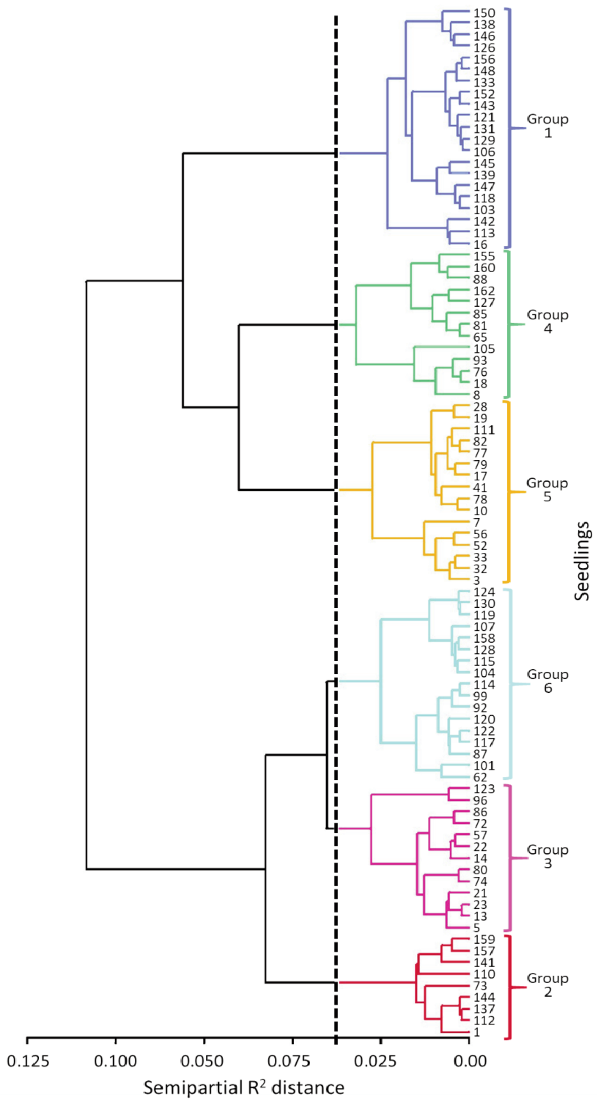

Fig. 4 - Cluster by the Ward method into six groups formed by the anatomical and physiological characteristics of the stem and leaf of 'Colín V-33' avocado seedlings. The black dotted line represents the dendrogram cut according to Hotelling's pseudo-statistic t^2.

Woodward, 2003). Consequently, these characteristics can be considered as representative of the adaptation to the environment of the plant, as a constant feature of taxonomic value, that the relationship between these two anatomical variables (epidermis thickness and stoma length) have with the variable air temperature.

The CC 3 was associated with the variables thickness of the cambium, transpiration rate, and stomatal conductance. Stomatal conductance refers to the control exerted by stomata on the rate of transpiration, representing the ability of a water molecule to diffuse through the leaf per unit of time. A minor stomatal conductance may be the expanation for lower CO_2 assimilation and at the same time a lower transpiration rate (Sholefield *et al*., 1980; Barrientos-Villaseñor *et al*., 1999).

Stomata size is considered a key physiological variable on stomatal conductance (Holland and Richardson, 2009), which is why the stomatic conductance use can be considered as a rapid test in genotype selection, as an indirect measure of stomatic density, since this can also be a way to perform a genotype separation towards dwarf types as proposed by Barrientos-Pérez and Sánchez-Colín (1987).

The *F* significance test of the Mahalanobis distance (Table 5) indicated that there are highly significant differences between the groups of plants at a level of $P<0.0001$.

To have a better and clearer visualization (distribution) of the groups of plants, the graphic representation was made in the first factorial plane with the first two canonical components (Fig. 5).

The graphic representation of the groups in the first factorial plane showed that the Group 4 is the only one that is isolated from the rest and was very different from the other five groups, with most of its

Table 2 - Groups formed according to the cluster analysis with 89 plants derived from 'Colín V-33' avocado seedlings, derived from anatomical and physiological characteristics of stem and leaf

Group	Seedings clustered
Group 1	16, 103, 106, 113, 118, 121, 126, 129, 131, 133, 138, 139, 142, 143, 145, 146, 147, 148, 150, 152, 156
Group 2	1, 73, 110, 112, 137, 141, 144, 157, 159
Group 3	5, 13, 14, 21, 22, 23, 57, 72, 74, 80, 86, 96, 123
Group 4	8, 18, 65, 76, 81, 85, 88, 93, 105, 127, 155, 160, 162
Group 5	3, 7, 10, 17, 19, 28, 32, 33, 41, 52, 56, 77, 78, 79, 82, 111
Group 6	62, 87, 92, 99, 101, 104, 107, 114, 115, 117, 119, 120, 122, 124, 128, 130, 158

Table 3 - Values of the canonical discriminant analysis of stem and leaf anatomical variables and physiological leaf variables of 89 'Colín V-33' avocado seedlings

Canonical component	Proper value	Variance (%)	Accumulated variance (%)	Canonical correlation	Approximated calculated *F*	*P>F*
1	47.504	39.91	39.91	0.909	4.93	<0.0001
2	34.405	28.90	68.81	0.880	4.02	<0.0001
3	18.683	15.69	84.50	0.807	3.09	<0.0001
4	13.703	11.51	96.01	0.760	2.45	<0.0001
5	0.4746	3.99	100	0.567	1.42	0.1419

Table 4 - Total canonical coefficients of the canonical discriminant analysis (CDA) of anatomical characteristics of the stem and leaf, as well of physiological characteristics of the leaf of 'Colín V-33' avocado seedlings. The highest values are presented in bold letters in three canonical components

Studied variable	Can 1	Can 2	Can 3	Can 4	Can 5
Epidermis (µm)	0.166274	0.677758	0.107810	-0.136872	0.076201
Parenchyma (µm)	0.250557	0.442521	-0.254777	-0.069388	0.232325
Phloem fibers (µm)	0.431579	0.577674	0.439686	-0.030758	0.020814
Phloem (µm)	0.432130	-0.365082	0.103285	0.028883	0.067597
Cambium (µm)	0.136514	-0.145255	0.545261	0.168825	-0.111704
Xylem (µm)	0.408444	-0.551603	-0.038947	0.244313	-0.159389
Pith (µm)	0.061974	0.521382	0.194067	0.087974	-0.233069
Stem Ø (µm)	0.601144	-0.122358	0.233650	0.369948	-0.235378
Vessel density by area (vessels/µm^2)	-0.642113	0.285704	-0.040889	-0.295962	0.234301
Vessel area (µm^2)	0.198248	0.296278	0.035828	0.557918	0.230066
Mayor axis of vessel (µm)	0.540765	-0.053647	-0.094319	0.682230	0.145237
Minor axil of vessel (µm)	0.464509	0.339487	0.238300	0.596176	0.043128
Wall thickness of two cells (µm)	0.262160	0.553793	0.219939	0.168747	-0.051240
Roundness index	0.521700	-0.516643	-0.056714	0.381362	-0.115487
Feret Ø	-0.415188	0.247956	-0.183468	-0.143826	0.263973
Leaf temperature (°C)	-0.055325	0.671497	0.197631	-0.080977	0.002190
CO_2 assimilation rate (µmol·m^{-2}·s^{-1})	-0.346322	0.209754	-0.090718	0.653934	-0.383860
Transpiration rate (mmol·m^{-2}·s^{-1})	-0.538189	0.110232	0.546988	0.242327	0.023917
Stomatal conductance (mmol·m^{-2}·s^{-1})	-0.530059	-0.016900	0.502987	0.237708	0.022604
Internal CO_2 concentration (ppm)	-0.035307	-0.155754	0.408420	-0.518668	0.491616
WUE	0.010946	0.092531	-0.434341	0.556483	-0.488497
Stomatal density (stomata/mm^2)	0.059033	-0.043825	0.165589	0.198190	0.023354
Epidermis cell density (cells/mm^2)	-0.032255	0.110568	0.025203	-0.166117	0.273310
Stomatal index (%)	0.098199	-0.132837	0.100880	0.400103	-0.233939
Stoma length (µm)	0.087852	0.636119	-0.014346	-0.140672	-0.173689

WUE= Water use efficiency index.

plants in the lower left quadrant (Fig. 5).

Groups 1, 2 and 6 are probably clustered because they are plants with anatomical and/or physiological similarities according to the variables evaluated in this study (Fig. 5). These groups are those that have dispersion in both planes, so they are considered the most heterogeneous groups within the plants studied.

Groups 3 and 5 were slightly more separated from the rest of the groups, but between them, there is some closeness (less dispersion), which could be inferred as similar groups according to the behavior of the variables evaluated (Fig. 5).

Table 5 - Mahalanobis distance and its significance, from anatomical characteristics of stem and leaf, as well of physiological characteristics of leaf from 89 'Colín V-33' avocado seedlings

Group	1	2	3	4	5	6
1	-					
2	18.20	-				
3	24.83 ***	36.28 ***	-			
4	37.84 ***	39.65 ***	55.11 ***	-		
5	22.39 ***	34.87 ***	16.52 ***	29.83 ***	-	
6	12.92 ***	22.02 ***	16.19 ***	36.78 ***	23.52 ***	-

*** Significant at P<0.0001 of probability.

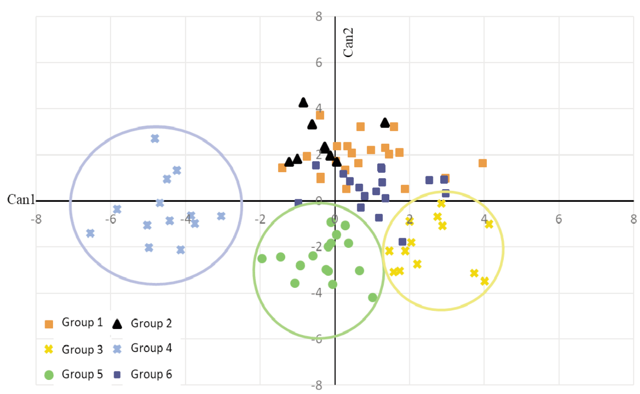

Fig. 5 - Dispersion of the six groups of 'Colín V-33' avocado seedlings in the first factorial plane of the first two canonical components of the Canonical Discriminant Analysis, derived from anatomical and physiological characteristics of stem and leaf.

In the pattern of dispersion of the plants in a three-dimensional graph formed by the three canonical components (CC), it was observed that group 4 is completely separated from the rest and is located towards the most negative values of CC 1 and CC 3 (Fig. 6), it was also observed that groups 3, 5 and 6 were not so separated from the rest of groups (less dispersed), such as 4, and if they could be easily differentiated in the graph located towards positive values of CC 1 and CC 2; on the other hand, the most dispersed groups were 1 and 2, mixing with each other, indicating that they probably have similar characteristics.

Group 1 is formed by plants with the greatest epidermis thickness and the greatest stoma length but the lowest stomatal conductance of all other groups (Fig. 6). The thickness of the epidermis is a characteristic that may be related to drought tolerance according to Baas (1982), as it is the most exposed anatomical characteristic of the plant along with the stomatic density, representing the last link of the transpiration stream into the atmosphere (Faust, 1989).

The plants of group 2 were characterized by having the cambium with less thickness, high average values of stem diameter and stomata length.

Group 3 was characterized by having plants with greater stem diameter, a lower density of vessel elements and shorter stomata length, low average values in transpiration and stomatal conductance. This group of plants is likely to be more vulnerable to physiological problems such as drought as reported by Reyes-Santamaría *et al*. (2002).

The plants in group 4 had the lowest values for stem diameter, but the highest density of xylem vessel elements, also the highest rate of transpiration and the highest stomatal conductance, this being the group that separated completely from the rest (Fig. 4). The results could indicate that the plants belong-

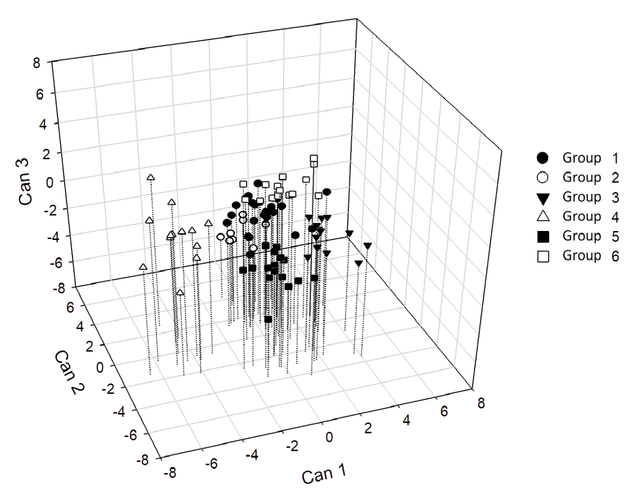

Fig. 6 - Dispersion of 89 'Colín V-33' avocado seedlings in the first three canonical functions based on physiological and anatomical variables of leaf and stem.

ing to this group probably use more water and can be more adapted to condition of more availability, because by decreasing the stomatal opening and closing it contributes to reducing the loss of water due to evapotranspiration (Núñez-Colín *et al*., 2006). Previous results (Sholefield *et al*., 1980; Barrientos-Villaseñor *et al*., 1999) indicated that there is a positive correlation between the variables CO_2 assimilation, stomatal conductance and transpiration rate, thus an increase in stomatal conductance promotes the increase in the transpiration rate and the photosynthetic rate (Damián-Nava *et al*., 2009).

Group 5 was represented by plants with the lowest thickness of the epidermis, the lowest leaf temperature, low stomatal conductance values, and transpiration. Group 6 (Fig. 5) was characterized by having the greater cambium thickness, high average values in the variables stem diameter, epidermis thickness, leaf temperature, stomata length, but low densities values of vessel elements, transpiration, and stomatal conductance.

Groups 1 and 6 were closest to each other, according to the Mahalanobis distances (Table 4), while the most distant groups were 3 and 4 (Fig. 5).

For any type of plant, the conducting tissue of the stem, the size of the xylem vessel elements, the percentage of the xylem and phloem, and the relationship between xylem and phloem, are anatomical features that define the transport capacity of water in plants. It has been observed in some tree species, that as the percentage of vascular tissues increases and the diameter of the vessels are smaller, the amount of water transported is higher, and this may be an indicator of greater adaptation from plants to low humidity conditions in the soil (Vasconcellos and Castle, 1994; Reyes-Santamaría *et al*., 2002). For the case of avocado rootstocks, it has been found that stems of 'Duke 7' (1248.7 μm^2) had narrowed xylem element cells diameters than 'Toro Caynon' (1536.1 μm^2), were 'Duke 7' showed higher daily sap flow (2.8 kg day^{-1}) compared to 'Toro Caynon' (2.0 kg day-1) on ungrafted plants and in the case of grafted plants it also increased the sap flow when 'Duke 7' was used (Fassio *et al*., 2009).

Several authors compared normal (vigorous) trees with dwarf trees of citrus, olive, mango, and *Copaifera langsdorfi* found that dwarf trees are characterized by narrow (small) vessel elements and a higher density of xylem vessel elements (Saeed *et al*., 2010; El Said *et al*., 2013; Rashedy *et al*., 2014; Longui *et al*., 2014, respectively) which has also been found in avocado (Reyes-Santamaría *et al*., 2002). The results found showed that the plants of Group 4 are the ones that had the highest densities of vessels and with this, probably less vessel element diameter, increasing transport and conferring a low probability of suffering cavitation and embolism (Núñez-Colín *et al*., 2006). On the other hand, Goncalves *et al.* (2007) and Tombesi *et al.* (2010) indicated that in cherry and peach dwarf rootstocks, respectively, the diameter of xylem vessel elements is smaller. It has been found in peach that large diameters of xylem vessel elements are found in the more invigorating rootstocks (Bruckner and DeJong, 2014). For all the above, it is inferred that genotypes belonging to Group 4 are small size plants or with dwarfing characteristics, this statement is reinforced by studies that showed that intermediate and small size plants frequently have a smaller stem diameter, such as the case of the Group 4 plants that had the lowest average stem diameter values (Fig. 7). These results show a similar trend to the study of López Jiménez and Barrientos Priego (1987) in trees of 'Colín V-33' where the trunks in dwarf trees had a circumference and average diameter smaller than the tall trees.

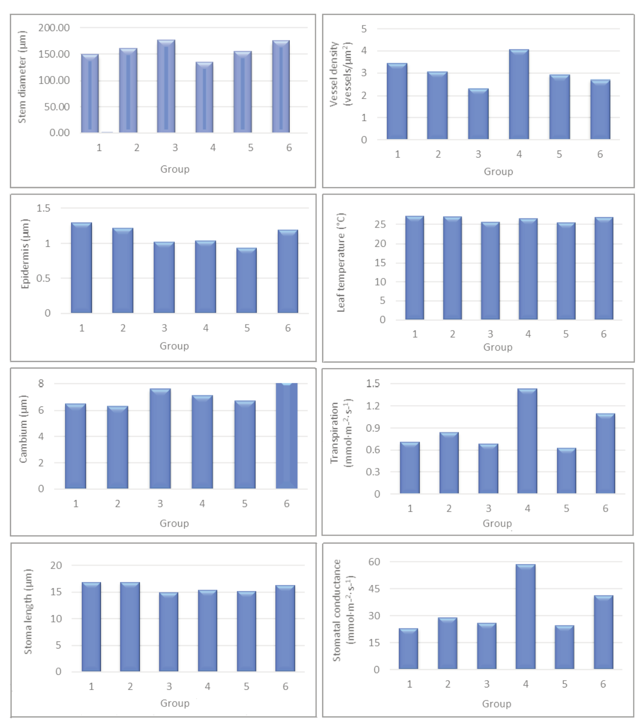

Fig. 7 - Discriminant variables of plants derived from 'Colín V-33' avocado seedlings in the six groups generated according to canonical discriminant analysis (ADC) and Ward cluster, derived from anatomical and physiological characteristics of stem and leaf.

The classification results show that 97.8 % of originally grouped individuals were correctly categorized (Table 6), which indicated that the six groups based on this are consistent, showing the stability of belonging to each group. Where only two individuals were atypical of their group. These results give certainty to the analysis performed and the congruence of the groups obtained.

4. Conclusions

The variables stem diameter, the density of vessel elements, thickness of the epidermis, leaf temperature, stomata length, thickness of the cambium layer, transpiration rate and stomatal conductance, discriminated the groups of 'Colín V-33' avocado seedling plants correctly.

The main correlations obtained were a positive correlation between the density of xylem vessel elements with the rate of transpiration and with stomatal conductance. These correlations indicated a linear relationship of these variables and this last can be used as an index of preselection to discriminate the vessel element density in seedlings.

A higher density of elements of xylem vessel elements and smaller stem diameter appear to be indicators of dwarf plants in avocado, so these characteristics could allow the selection of genotypes with behavior of this type of growth.

Group 4 genotypes presented anatomical and physiological characteristics probably associated with dwarf types unlike Group 3, which were plants with typical anatomical and physiological characteristics of vigorous plants.

To demonstrate the use of the different contrasting groups it is required further studies with grafted plants to determine their potential according to their rootstock characteristics.

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Table 6 - Summary of a priori resubstituting test using the linear discriminant function of 'Colín V-33' avocado seedlings and formed by anatomical and physiological characteristics of the stem and leaf

	Number of observations and percentage of classified by group						
Group	1	2	3	4	5	6	Total
1	20*	1	0	0	0	0	21
%	95.24	4.76	0.00	0.00	0.00	0.00	100
2	0	9*	0	0	0	0	9
%	0.00	100	0.00	0.00	0.00	0.00	100
3	0	0	12*	0	0	1	13
%	0.00	0.00	92.31	0.00	0.00	7.69	100
4	0	0	0	13*	0	0	13
%	0.00	0.00	0.00	100	0.00	0.00	100
5	0	0	0	0	16	0	16
%	0.00	0.00	0.00	0.00	100	0.00	100
6	0	0	0	0	0	17*	17
%	0.00	0.00	0.00	0.00	0.00	100	100
Total	20	10	12	13	16	18	89
%	22.47	11.24	13.48	14.61	17.98	20.22	100

*97.8 % of individual grouped and correctly classified.

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