



Article

# Root Systems of Five Clonal Avocado Genotypes

Nancy Elena Gonzalez-Florez <sup>1</sup>, Alejandro Facundo Barrientos-Priego <sup>1</sup>, \*, Eduardo Campos-Rojas <sup>1</sup>, María Teresa Beryl Colinas y León <sup>1</sup> and Prometeo Sánchez García <sup>2</sup>

- Departamento de Fitotecnia, Universidad Autónoma Chapingo, Km. 38.5 Carretera, México-Texcoco, Texcoco 56230, MX, Mexico; nancyegonzalezf@gmail.com (N.E.G.-F.); ecamposr@chapingo.mx (E.C.-R.); mcolinasy@chapingo.mx (M.T.B.C.y.L.)
- Especialidad de Edafología, Colegio de Posgraduados, Km. 36.5 Carretera, México-Texcoco, Texcoco 56264, MX, Mexico; sanchezgarciaprometeo@gmail.com
- \* Correspondence: abarrientosp@chapingo.mx; Tel.: +52-5959521616

Abstract: The root system of clonal rootstocks has been poorly studied, despite its crucial importance. Roots not only provide support and nutrition to the plant but also contribute to tolerance to pests, diseases and environmental stresses, in addition to optimizing yields. Although the initial cost of clonal rootstocks is higher, the investment is offset by the reduction in phytosanitary treatments, greater longevity of the trees and a lower mortality rate. The aim of this research was to quantify the root system growth of five clonally propagated dwarf and normal avocado genotypes evaluated in rhizotrons, with the perspective of identifying distinctive characteristics suitable for their possible use in container culture. The avocado clonal plants to be evaluated were placed in rhizotrons and evaluated for six months, where 35 growth variables were evaluated with the aid of a root analyzer program. A randomized complete block design with five treatments (genotypes) and three replications was used under greenhouse conditions. Analysis of variance, variable purge and multivariate analysis were performed. It was found that 'Duke 7' and 'San Martín' were statistically different for most of variables, with 'San Martín' showing less root growth and 'Duke 7' showing remarkable lateral growth. The adventitious root system's growth depends on the genotype, and the use of rhizotrons allows its study, which proved to be a useful methodology for this type of evaluation since it allows the visualization and adequate quantification of root growth. Genotypes with less root growth may be suitable for use in container culture, and roots with extensive lateral growth may be useful in shallow soils.

Keywords: Persea americana Mill.; dwarf avocado; clonal rootstocks; root growth



Academic Editor: Elena Baldi

Received: 27 December 2024 Revised: 6 February 2025 Accepted: 12 February 2025 Published: 21 February 2025

Citation: Gonzalez-Florez, N.E.; Barrientos-Priego, A.F.; Campos-Rojas, E.; Colinas y León, M.T.B.; Sánchez García, P. Root Systems of Five Clonal Avocado Genotypes. *Horticulturae* 2025, 11, 232. https://doi.org/ 10.3390/horticulturae11030232

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

# 1. Introduction

Most commercial avocado plantations use rootstocks originating from seeds [1]. Although 'criollo' seedling rootstocks offer high adaptability, they have considerable genetic variability, which does not guarantee stable behavior in the field [2]. This situation went unnoticed by the industry until a few years ago, when plantations were established in good quality soils. However, market and growing conditions have changed, moving the crop to areas with edaphic limitations [1], which mainly affect the young roots responsible for nutrient uptake and growth [3]. Therefore, the development of new avocado rootstocks is of great relevance.

There are several breeding programs for avocados (*Persea americana* Mill.) focused on developing new cultivars and rootstocks to increase productivity and address soil stress

problems [2]. Other programs focus on incorporating disease resistance and other desirable traits [4]. However, it is not always possible to bring together all desired traits in a single rootstock [5]. Recently, there has been an increase in research on avocado rootstocks, an area previously explored little due to the complexity and time required for this type of study [6], where clonal propagation is vital to maintain the relevant characteristics of the rootstock.

The current trend in fruit tree propagation is rooted rootstocks, which eliminate genetic variability and reduce the production onset [7]. Only rootstocks obtained by asexual methods are genetically identical to the mother plant [8]. Selecting and obtaining these clonal avocado rootstocks is one of the greatest challenges in modern fruit growing, since their propagation is a long and costly process [4,9]. According to Barrientos-Priego et al. [10], the ideal avocado tree should have a maximum height of 5 m, which would facilitate phytosanitary control, harvesting, pruning and foliar fertilization. This type of tree would allow the establishment of higher-density plantations, increasing the production per unit area. An alternative for avocado cultivation is establishment in containers, which has been used in some studies on water use and shown that it is possible to obtain production under such conditions [11]. In Chile, it has been proposed as an alternative in greenhouse systems, where it could help mitigate the impact of climate change and reduce its water footprint by having a controlled system [12]. In these alternative systems, dwarfing rootstocks can play an important role.

In the case of avocados, there are some dwarf genotypes which possibly have dwarfing characteristics for their rootstocks. In other fruit trees, such as apples, specific genes have been located in rootstocks which contribute to the tree by reducing its size when grafted with normal-growing varieties [13]. In the case of avocados, these genes have not been studied, and it is also necessary to evaluate them when using them as rootstocks, in addition to studying their root systems.

The root system of clonal avocado trees is shallow, with a greater number of fine roots and a greater length density [14]. Several authors have suggested that the size and volume of the root system are important criteria for estimating plant quality and predicting plant performance in the field after transplanting. Plants with more developed root systems overcome transplanting stress better, show higher growth potential and improved water and nutrient uptake [15].

Roots, which are responsible for anchoring and mechanically supporting the aerial part of a plant in addition to absorbing water and nutrients, are a little-studied organ despite their importance [16]. It is essential to know the quantity and distribution of roots in the soil profile [17]. Furthermore, characterizing roots according to their branching order and anatomy is a useful approach for identifying functional differences within and between root systems [14], because these systems vary widely in extent and distribution in the soil profile [17].

Techniques such as using rhizotrons make it possible to observe root growth in situ and obtain a realistic picture of its distribution and intensity within the rhizosphere [18]. Rhizotrons are devices which allow studying the root systems of plants as they are designed with two glass sides, allowing observation of the growth and development of the root system from a noninvasive and non-destructive perspective. However, there is little research describing the variation in root architecture of fruit crops [19]. Due to the complexity of the study of roots in fruit trees, this topic has not been sufficiently investigated. This study, which used the rhizotron as a tool, aimed to generate information on the root growth of dwarf genotype rootstocks. This could allow a better understanding of root system conformation and facilitate their possible use as rootstocks based on these characteristics.

## 2. Materials and Methods

This research was carried out in a greenhouse of the Department of Phytotechnology at the Experimental Field of the Universidad Autónoma Chapingo, located in Chapingo, State of Mexico.

#### 2.1. Plant Material

Five avocado clonal genotypes were selected for their dwarf growth habits and compared to a normal growth genotype (control) used as the world standard (Table 1).

Table 1. Avocado genotypes studied and their root system characteristics.

Genotype	Origin and Race	Characteristic	
'Duke 7'	California, USA. 'Duke' seedling, Mexican race.	Normal growth with medium-length internodes, tall size tree.	
'San Martín'	Tezuitlán, Puebla. Mexico. Mexican race seedling.	Dwarf growth with rather short internodes, extremely small tree.	
'Colín V-33'	Ixtapan de la Sal, Estado de México, Mexico. 'Fuerte' seedling, hybrid Mexican-Guatemalan race.	Dwarf growth, medium-length internodes, lateral extensive branch growth, loss of apical dominance, small tree.	
'Misterioso'	Coatepec Harinas, Estado de México, Mexico. 'Frazer' seedling, hybrid Mexican-Guatemalan race.	Dwarf growth with rather short internodes, extremely small tree.	
'Fundación II'	Coatepec Harinas, Estado de México, Mexico. 'Hass' seedling, hybrid Mexican-Guatemalan race.	Dwarf growth, medium-length internodes, lateral extensive branch growth, loss of apical dominance, small tree.	

The cloning method used to obtain the plants was based on the work of Frolich and Platt [20], which is used worldwide to clone avocado rootstocks. The procedure consisted of producing an avocado plant from seed and, when it reached the appropriate diameter, a simple cleft grafting of the rootstock to the root. After complete union of the graft, the plants were placed in a dark chamber at 30  $^{\circ}$ C to obtain etiolated shoots from the grafts. When these reached a length of approximately 15–20 cm, the plants were removed from the dark chamber and transferred to an illuminated greenhouse. At the base of the etiolated shoot, potassium salt of indole-3-butyric acid (Sigma-Aldrich; San Luis, MO, USA) was applied at 7000 mg L $^{-1}$  at a pH of 7 after placing a container at the base of the shoot and using coconut peat (Hydro Enironment S.A. de C.V.; Tlaneplantla, Mexico) as a rooting medium.

## 2.2. Experimental Establishment

Based on previous studies carried out on the root system of peach seedlings using rhizotrons by Lesmes-Vesga et al. [19], it was decided to use this method to evaluate the root growth of the clonal avocado rootstocks. The rhizotron was  $40~\rm cm \times 40~\rm cm \times 2.5~\rm cm$  in length and had two transparent glass plates ( $40~\rm cm \times 40~\rm cm$  in length) joined on both sides by a three-sided wooden frame with a perforated bottom to allow water filtration. The rhizotrons were filled with river sand (4 L), and the clonal plants were transplanted into them, corresponding to each treatment. Both sides of the rhizotron were covered with black and white bicolor polyethylene (Agripolyane – Tecnologías Agrícola del Centro S.A. de C.V., Tulancingo de Bravo, Mexio) with the white side facing out to avoid light and keep the roots in dark conditions. The experiment was conducted under greenhouse conditions

Horticulturae **2025**, 11, 232 4 of 15

without temperature control, with an average temperature of 17 °C and a relative humidity of 71.9%. A drip fertigation system was used with two emitters calibrated to deliver 4 L per hour each, with a watering frequency of two minutes every 24 h and, in winter, every 48 h throughout the experiment, as water requirements were lower in the latter period due to lower temperatures. The nutrient formula supplied in mg  $L^{-1}$  was as follows: 59 (N), 11 (P), 96 (K), 63 (Ca), 17 (Mg), 39 (Fe), 0.025 (Cu), 0.5 (Mn), 0.05 (Zn) and 0.5 (0.5).

## 2.3. Root System Monitoring

Root systems were photographed in the rhizotrons from each side of the glass windows once every two weeks for six months to assure good time for root growth, and graphs were created to visualize the root behavior. After this period, the roots were extracted for measurement by removing one of the rhizotron crystals. A nail board was used to extract each plant's root system to maintain the spatial distribution of the roots. The nail board consisted of an acrylic panel with 2.5 cm-long nails separated by 2.5 cm  $\times$  2.5 cm in length, and the substrate was separated from the roots inserted into the nail board by gently spraying water. Once the root systems were extracted from the rhizotrons, photographs were taken.

## 2.4. Image Analysis and Measurement Program

From the digital photographs taken every two weeks, the depth and width reached by the complete root system were measured, and the depth/width ratio was estimated with the images, which were processed with ImageJ version 1.48v software [21]. These two variables were also quantified for the bare root in order to have data in this condition.

A search of available root system analysis software was conducted, and at least 12 programs were explored, finding that RhizoVision Explorer version 2.0.3 [22] was suitable, easy to use and free to use. Digital photographs obtained at the end of the experiment (bare root system) were analyzed with the root image analysis software RhizoVision Explorer. By default, the software provides data for 35 variables which are used for the statistical analysis as variables (Table 2).

**Table 2.** Root variables evaluated in cloned avocado genotypes according to RhizoVision Explorer software. More details of each variable can be found in the manual (https://www.rhizovision.com/manual; accessed on 17 February 2025) or in the work of Seethepalli and York (2020) [22].

	Abbreviation	Variable
1	MinNR	Median Number of Roots
2	MaxNR	Maximum Number of Roots
3	NRT	Number of Root Tips
4	TRL	Total Root Length (mm)
5	Depth	Depth (mm)
6	$M\widetilde{W}$	Maximum Width (mm)
7	WDR	Width-to-Depth Ratio
8	NA	Network Area (mm <sup>2</sup> )
9	CA	Convex Area (mm <sup>2</sup> )
10	S	Solidity
11	LRA	Lower Root Area (mm <sup>2</sup> )
12	AD	Average Diameter (mm)
13	MedD	Median Diameter (mm)
14	MaxD	Maximum Diameter (mm)
15	P	Perimeter (mm)
16	Vol	Volume (mm <sup>3</sup> )
17	SA	Surface Area (mm²)

Horticulturae **2025**, 11, 232 5 of 15

Table 2. Cont.

	Abbreviation	Variable
18	Н	Holes
19	AH	Average Hole Size (mm <sup>2</sup> )
20	ARO	Average Root Orientation
21	SAF	Shallow Angle Frequency
22	MAF	Medium Angle Frequency
23	SteepAF	Steep Angle Frequency
24-26	RLDR 1–3 layer	Root Length Diameter (mm)
27-29	PADR 1–3 layer	Projected Area Diameter (mm <sup>2</sup> )
30-32	SADR 1–3 layer	Surface Area Diameter (mm <sup>2</sup> )
33–35	VDR 1–3 layer	Volume Diameter (mm <sup>3</sup> )

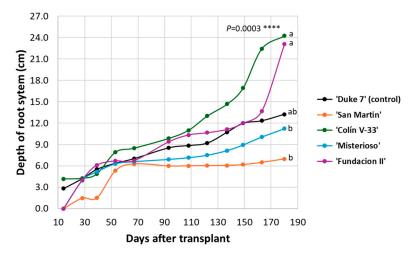
# 2.5. Replications, Experimental Design and Statistical Analysis

The experiment design used was a randomized complete block according to the pressurized irrigation system, with five treatments and three replicates. The resulting data of the variables were compared between treatments with analysis of variance, standard error and Tukey's mean comparisons ( $p \leq 0.05$ ) with SAS statistical software version 9.4 [23]. The variables in Table 2 were depurated according to López-Santiago et al. [24], as procedures were carried out to eliminate highly associated variables with no differences, assuring correct identification of the principal variables contribute to the distinctness of a multivariable analysis. Subsequently, a principal component analysis was performed, in addition to neighbor-joining clustering analysis and Manhattan distance analysis [25] with 10,000 resamples using the PAST 3.26 program [26]. This resampling (bootstrap) allowed us to have a better idea of the clusters formed and gave us a robust dendrogram with a high probability of occurrence.

#### 3. Results

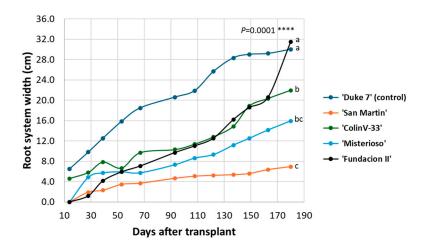
## 3.1. Follow-up of Complete Root System in Rhizotron

The root systems showed significant differences at the end of monitoring in the rhizotrons in terms of depth in the case of 'Colín V-33' and 'Fundación II' with respect to 'Misterioso' and 'San Martín' (Figure 1). 'San Martín' showed a notorious root system with less depth.



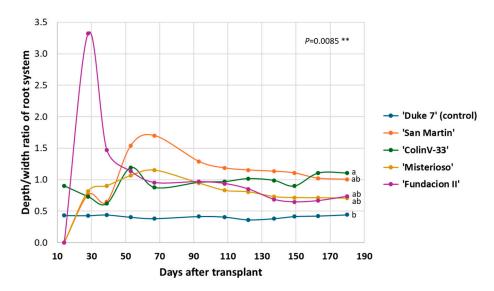
**Figure 1.** Depths of the adventitious root systems of avocado genotypes, obtained from the monitoring of rhizotrons using digital photographs. Letters for the last day indicate different values according to Tukey's test at  $p \le 0.05$ . \*\*\*\*  $p \le 0.001$  significance according to the variance analysis.

With respect to the final width (Figure 2), 'San Martín' showed the smallest width and was significantly different from 'Fundación II', 'Duke 7' and 'Colín V-33' but not significantly different from 'Misterioso'.



**Figure 2.** Widths of the adventitious root systems of avocado genotypes, obtained from monitoring rhizotrons using digital photographs. Letters for the last day indicate different values according to Tukey's test at  $p \le 0.05$ . \*\*\*\*  $p \le 0.001$  significance according to the variance analysis.

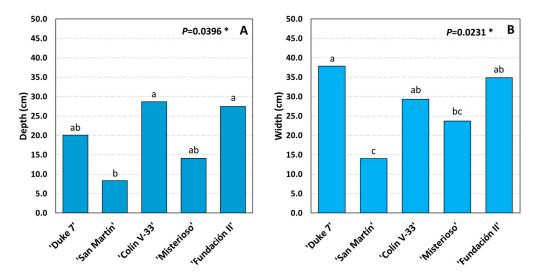
The width of 'Fundación II' and 'Duke 7' had significant differences with 'Colín V-33' and 'Misterioso'. For the depth/width ratio (Figure 3), significant differences were found only between 'Colín V-33' and 'Duke 7', showing a uniform ratio during all of the evaluation. It should be noted that monitoring in rhizotrons generates estimates of root system growth which may differ from the total root system, since not everything can be quantified.



**Figure 3.** Depth/width ratios of the adventitious root systems of avocado genotypes, obtained from monitoring rhizotrons using digital photographs. Letters for the last day indicate different values according to Tukey's test at  $p \le 0.05$ . \*\*  $p \le 0.001$  significance according to the variance analysis.

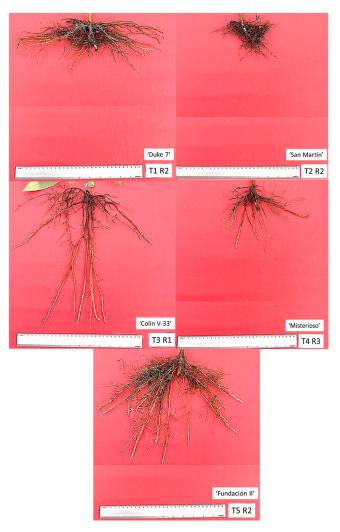
#### 3.2. Complete Root System Outside the Rhizotron

'Colín V-33' and 'Fundación II' showed the deepest root systems (Figure 4A), with no significant differences with 'Duke 7' and 'Misterioso'. 'San Martín' had the lowest depth. The greatest width was found for 'Duke 7' (Figure 4B), showing significant differences with 'San Martín' and 'Misterioso'.



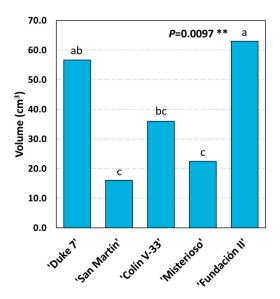
**Figure 4.** (**A**) Depths and (**B**) widths of the adventitious root systems of avocado genotypes, obtained after extraction of rhizotrons. Letters indicate different values according to Tukey's test at a  $p \le 0.05$ . \*  $p \le 0.01$  significance according to the variance analysis.

These differences can be seen visually in the corresponding photographs (Figure 5).



**Figure 5.** Adventitious root systems of avocado genotypes after removal from rhizotrons. Letters T and R represent treatment (genotype) and replicate, respectively. Scale = 30 cm.

The root systems showed significant differences at the end of monitoring in the rhizotrons in terms of volume (Figure 6); 'San Martín' and 'Misterioso' had the lowest volumes, which were significantly different from 'Fundación II' and 'Duke 7'.



**Figure 6.** Volumes of the adventitious root systems of avocado genotypes obtained after extraction from rhizotrons. Letters indicate different values according to Tukey's test at  $p \le 0.05$ . \*\*  $p \le 0.001$  significance according to the variance analysis.

It should be noted that the two genotypes with short internodes and dwarfs, 'San Martin' and 'Misterioso', had smaller root systems with limited exploration in the substrate. While the dwarfs with extensive lateral growth had root systems with greater exploration in terms of depth, the control 'Duke 7' with normal growth had merely lateral growth and limited depth exploration. Those differences can be appreciated in Figure 5.

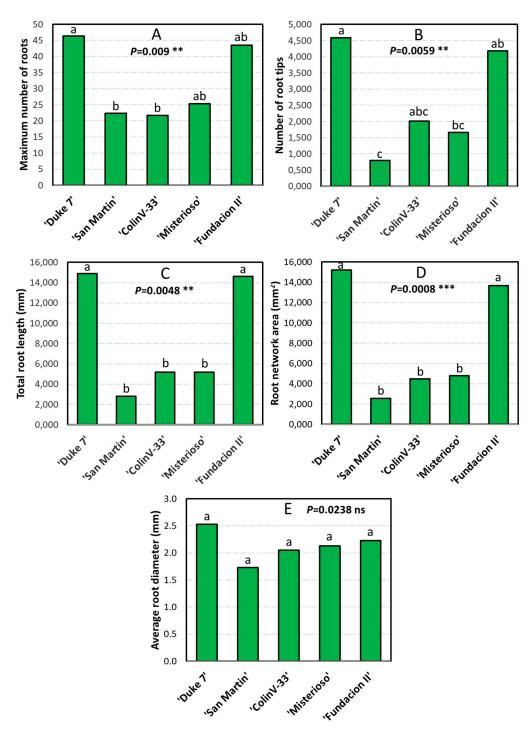
# 3.3. Main Variables of Root Growth Through Image Analysis

'Duke 7' and 'Fundación II' exhibited higher values for the maximum number of roots (Figure 7A), number of root tips (Figure 7B), total root length (Figure 7C) and root network area (Figure 7D), while 'San Martín' showed lower values for most of the variables. For the variable average root diameter, there were no significant statistical differences (Figure 7E).

# 3.4. Depuration and Principal Component Analysis (PCA) of Root Growth Variables

After variable depuration, the following variables were found: the maximum number of roots, root depth (mm), maximum root system width (mm), root network area (mm<sup>2</sup>), root system solidity, average root diameter (mm) and projected root diameter in layer 2 (mm). These were included in the PCA as well in the cluster analysis.

In the PCA, with the resulting variables after depuration, the first principal component explained 99.8% of the total variance (Table 3), and the values of the principal variables comprising these components were the root network area (mm²) and projected root diameter in the second layer (mm) for CP 1 and the projected root diameter in the second layer (mm), root network area (mm²) and root depth (mm) for CP 2 (Table 4).



**Figure 7.** Morphological variables of the adventitious root system of avocado genotypes after removal from the rhizotrons: (**A**) maximum number of roots, (**B**) number of root tips, (**C**) total root length, (**D**) root network area and (**E**) average root diameter. Letters indicate different values according to Tukey's test at a  $p \le 0.05$ . ns: not significant, \*\*  $p \le 0.001$ , \*\*\*  $p \le 0.0001$  significance according to the variance analysis.

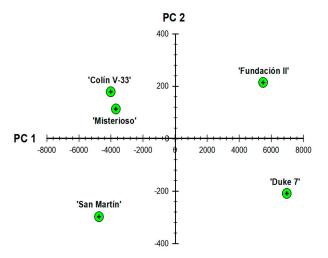
**Table 3.** Eigen values and accumulated variance of principal component analysis of avocado genotypes adventitious root system variables. Obtained after rhizotron extraction and image analysis.

Principal Component	Eigen Value	Variance (%)
1	$3.27 \times 10^{7}$	99.821
2	55,768.3	0.17004
3	1877.25	0.0057237
4	930.56	0.0028373

**Table 4.** Eigen values of the variables which formed the first two principal components of adventitious root systems of avocado genotypes. Obtained after rhizotron extraction and image analysis.

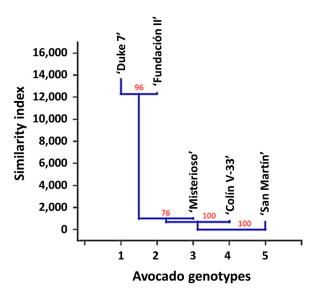
Variable	PC 1	PC 2
Maximum number of roots	0.0021239	-0.0002322
Root depth (mm)	0.0046799	0.20023
Maximum broadness of root system (mm)	0.013135	0.039346
Area of root network (mm <sup>2</sup> )	0.96847	-0.24493
Solidity of root system	$1.58 \times 10^{-6}$	-0.0003678
Average diameter of roots (mm)	$-3.64 \times 10^{-7}$	-0.0019959
Projected diameter of roots in the second layer (mm)	0.24873	0.94782

In the two-dimensional projection of avocado genotypes for the first two principal components (Figure 8), four groups were defined. The first group consisted of 'Fundación II' in the positive plane, 'San Martín' in the negative plane, 'Duke 7' in the positive-negative plane and 'Colín V-33' and 'Misterioso' in the negative-positive plane.



**Figure 8.** Dispersion of avocado genotypes in the first factorial plane of the first two functions of the principal component analysis (PC), derived from adventitious root system characteristics. PC 1: Root network area (mm<sup>2</sup>) and root diameter in the second layer (mm). PC 2: Projected root diameter in the second layer (mm), root network area (mm<sup>2</sup>) and root depth (mm).

The clustering analysis showed a remarkable similarity between the genotypes 'Duke 7' and 'Fundación II', which clustered closer together and further away from the rest of the genotypes (Figure 9). In contrast, 'San Martin' was located in a basal branching, indicating a greater distance from the others. This result agrees with and reinforces the findings in the principal component analysis, since most of the branches had high bootstrap values, making the analysis more robust.



**Figure 9.** Clustering of avocado genotypes with the neighbor-joining method and Manhattan distance with 10,000 resamples, derived from adventitious root system characteristics. The values in the branches are the probability as a percentage of occurrence according to the bootstrap.

Root network area and projected root diameter in the second layer of 'Duke 7' and 'Fundación II' were higher and different from the rest of the genotypes (Table 4), and in terms of root depth, 'Colín V-33' and 'Fundación II' showed the highest values and were not different from 'Duke 7' and 'Misterioso', while 'San Martín' was the one with the lowest value.

# 4. Discussion

The observed root system was generally shallow and extended at the time of measurement, which is in agreement with Castro et al. [16], who noted that avocado roots are shallow, lack root hairs and extend into the canopy projection zone. In contrast to what has been found in peach x almond backcrosses, in which their root system develops greater depth, which facilitates exploration of the sandy soil and allows access to water and nutrient availability [27], key aspects for an efficient root system. It has been indicated that varieties of fruit species with deep roots showed better growth and higher productivity [28]. The roots of the dwarf 'Colín V-33' and 'Fundación II' were deeper and highly contrasting with the control 'Duke 7', which showed contrasting shallow roots. Thus, the performance of these genotypes should be evaluated in field conditions to assure their root exploration distribution in the soil. However, avocado is characterized by having a root system which extends into the first 0.60 m of soil, although they can reach depths greater than 1.5 m [29]. Lynch [30], in this respect, pointed out that shallow roots are beneficial in humid climates, where they can effectively capture nutrients from the surface soil, as can be the case for 'Duke 7', which presented a shallow but extensive root system.

As for the average root diameter, no differences were observed, which contrasted with the findings of Atkinson and Wilson [31], who stated that as the root system matures, it develops roots of different ages and diameters, which improves its capacity to absorb water and nutrients. However, it should be emphasized that the growth of the root system was initial and determined by the age of the plants evaluated in the present study.

The width of the root system and its extension into the upper soil layers has been highlighted in sweet orange 'Pera' and can be effective, as long as the roots are horizontally extensive, which supports the vigor and yield of plants in rainfed conditions [32], a behavior which 'Duke 7' exhibited in our study, compared with other plants.

The adventitious root systems of the genotypes evaluated had contrasting differences, which has been observed in other species with respect to the architecture of the root systems, such as for peaches [19], and this influences nutrient absorption and mechanical stability [32]. This variability observed in avocado plants can be exploited to select types with greater root exploration and may be crucial for adaptation to specific soil conditions, which should be studied more extensively. It has been found that the adaptability of root systems to different soil types, such as in plantations of Cupressus funebris, underlines the need for a flexible approach in rootstock selection and management to optimize performance [33]. Similarly, in other species, it was highlighted that the remarkable variability in more complex root architecture traits suggests that it could contribute to their adaptability and productivity in different environments [34]. Containerized avocado cultivation opens up the possibility as an alternative for a production system under protected conditions to mitigate the impacts of climate change and the impact of the water footprint [12]. For this, selected rootstocks will be needed, perhaps one with the characteristics of limited root growth and exploration (as was found in our study), such as 'San Martín' and 'Misterioso', which are dwarf genotypes with short internodes, but this needs to be studied in the future.

On the other hand, Fassio et al. [6] noted that in clonal avocado trees, the absence of a taproot causes the root mass to develop well, with the main roots extending outward and downward, which are crucial for tree stability. Such a root mass was found for 'Duke 7' and 'Misterioso', with more roots and therefore more root tips, which may mean greater nutrient and water uptake as well as perhaps more synthesis of hormones such as auxins [35] and gibberellins [36]. This could also perhaps lead to better adaptation to rich soils, where minerals are available to be absorbed more efficiently.

It has been found that according to the method of rootstock propagation in citrus, the root architecture significantly influences the performance of grafted varieties [37]. In genetic studies on grapevine rootstocks, several quantitative trait loci (QTLs) associated with root characteristics have been identified, suggesting that certain genotypes could be selected and improved to optimize the root architecture and, consequently, increase productivity when grafted [38]. Since we found considerable variability in the avocados we studied, it is necessary to follow up on their grafted behavior and whether this difference in root systems is reflected by a grafted plant.

The plant root volume showed a positive correlation with the stem length and diameter as well as with the total biomass, and initial differences in plant size were maintained over time [15]. In this respect, both 'Duke 7' and 'Fundación II' showed larger root volumes, while the rest of the genotypes had smaller volumes. It has been suggested that root systems with robust root architectures are fundamental to maintaining productivity in trees [39], which can be the case for the two genotypes mentioned.

It has been highlighted that the main benefit of using clonal rootstocks in avocado lies in the superiority of producing roots and their growth, which positively impacts plant performance in the field [5]. In addition, studies have shown that 'Duke 7' clones have shown medium resistance to *Phytophthora cinnamomi*, higher productivity and fruit quality, as well as better resistance to edaphoclimatic stress [5]. The behavior of the root system of fruit trees evolves significantly over time, being characterized by distinct growth stages and variable architectures which adapt to conditions.

Principal component analysis allowed the identification of root system characteristics, which helped to discriminate between genotypes, as has been noted for other species [40]. This analysis indicated that the root network area and projected root diameter were in the second layer, with the former denoting a greater root system complexity and the latter regarding the distribution of the root system. The classification of the genotypes according to these main characteristics also coincided with the cluster analysis, which indicates the

usefulness of this type of multivariate analysis for selecting genotypes for their root system features and trying to determine their potential according to soil conditions [41] and soil types, as stated for other crops [42].

The relationship between the root system architecture and productivity in fruit trees is multifaceted and involves genetic, environmental and biological factors. While robust root systems generally correlate with higher productivity, specific interactions can vary significantly across genotypes and environmental conditions. Understanding these behaviors is essential for choosing a specific rootstock and succeeding in fruit tree cultivation.

The findings reinforce the importance of strategic selection based not only on immediate variability but also the potential for adaptation to different soil and climatic conditions, which is key in regions where efficient management of natural resources is a priority and in the context of a changing climate.

## 5. Conclusions

This study of root growth provided details on the architecture of the adventitious root systems of the avocado genotypes studied, where 'Duke 7' and 'San Martin' contrasted strongly for most of the variables studied and 'Fundación II' was similar to 'Duke 7', although with rather particular differences. Due to its root characteristics, 'Duke 7' can be suggested for use in shallow soils due to its strong lateral growth. The dwarf short internode genotypes 'San Martín' and 'Misterioso' could possibly be used for container cultivation due to their limited root systems. The dwarf genotypes 'Colín V-33' and 'Fundación II', on the other hand, had deeper roots and could explore the deeper soil layers.

The use of rhizotrons allowed the study of root systems in a controlled environment, showing that it is a useful tool for such studies, that it is possible to visualize and quantify root growth adequately and it can provide the possible use of a specific rootstock for certain types of cultivation, such as container cultivation or specific soil conditions. However, such a possibility needs to be verified under the relevant conditions as well as our results, especially the genotypes studied as grafted plants and their interactions.

**Author Contributions:** Conceptualization, N.E.G.-F. and A.F.B.-P.; methodology, A.F.B.-P., E.C.-R. and M.T.B.C.y.L.; software, A.F.B.-P.; validation, A.F.B.-P., M.T.B.C.y.L. and P.S.G.; formal analysis, N.E.G.-F. and A.F.B.-P.; writing—original draft preparation, N.E.G.-F. and A.F.B.-P.; writing—review and editing, N.E.G.-F., A.F.B.-P., E.C.-R., M.T.B.C.y.L. and P.S.G.; funding acquisition, A.F.B.-P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data are contained within the article.

**Acknowledgments:** N.E.G.-F. would like to thank the scholarship given by CONAHCyT. The authors thank the Fundación Salvador Sánchez Colín-CICTAMEX, S.C. for supplying some of the plant material used in the study and the Coordinación General de Estudios de Posgrado de la Universidad Autónoma Chapingo.

**Conflicts of Interest:** The authors declare no conflicts of interest.

# References

- Darrouy Palacios, N.; Castro Valdebenito, M.; Cautín Morales, R.; Kort Silva, L.; Bozzolo Artaza, R. Efecto de la posición de la yema y de la poda en plantas de aguacate destinadas a la clonación. Rev. Fitotec. Mex. 2010, 33, 249–256. [CrossRef]
- 2. González-Calderón, V.M.; Barrientos-Priego, A.F.; Núñez-Colín, C.A.; Ramírez-Ramírez, S.P.; Arpaia, M.L. Anatomía de la lámina de hoja en ocho cultivares de aguacate. *Rev. Mexicana Cienc. Agríc.* 2011, 2, 733–744. [CrossRef]
- Meza-Castillo, E.; Barrientos-Priego, A.F.; Rodríguez-Pérez, J.E.; Reyes-Alemán, J.C.; Borys, M.W.; Espíndola-Barquera, M.C. Caracterización morfológica e histológica de ápices de raíz de portainjertos de aguacate y su resistencia a factores adversos en el suelo. *Bioagro* 2018, 30, 107–116.

4. Ibarra-López, A.; Ojeda-Zacarías, M.D.; García-Zambrano, E.; Gutiérrez-Diez, A. Inducción *in vitro* de brotes de dos cultivares de aguacate raza Mexicana *Persea americana* var. *drymifolia* Schltdl. & Cham. *Rev. Mexicana Cienc. Agríc.* **2016**, 7, 337–347.

- 5. Freire Alberti, M.; Amaral Brogio, B.D.; Rodrigues da Silva, S.; Cantuarias-Avilés, T.; Fassio, C. Avances en la propagación de aguacate. *Rev. Bras. Frutic.* **2018**, 40, e-782.
- 6. Fassio, C.; Cautin, R.; Pérez-Donoso, A.; Bonomelli, C.; Castro, M. Propagation techniques and grafting modify the morphological traits of roots and biomass allocation in avocado trees. *Horttechnology* **2016**, *26*, 63–69. [CrossRef]
- 7. Lesmes-Vesga, R.A.; Chaparro, J.X.; Sarkhosh, A.; Ritenour, M.A.; Cano, L.M.; Rossi, L. Effect of propagation systems and indole-3-butyric acid potassium salt (K-IBA) concentrations on the propagation of peach rootstocks by stem cuttings. *Plants* **2021**, 10, 1151. [CrossRef]
- 8. Salazar-García, S.; Medina-Torres, R.; Ibarra-Estrada, M.E.; González-Valdivia, J. Influencia de portainjertos clonales sobre la concentración foliar de nutrimentos en aguacate 'Hass' cultivado sin riego. *Rev. Chapingo Ser. Hort.* **2016**, 22, 161–175.
- 9. Brokaw, W.H. Avocado clonal propagation. Proc. Int. Plant Prop. Soc. 1987, 37, 97–103.
- 10. Barrientos-Priego, A.F.; Muñoz-Pérez, R.; Reyes-Alemán, J.C.; Borys, M.W.; Martínez-Damián, M.T. Taxonomía, cultivares y portainjertos. In *El Aguacate y su Manejo Integrado*, 2nd ed.; Téliz, D., Mora, A.C., Eds.; Mundi Prensa: Distrito Federal, México, 2007; pp. 31–62.
- 11. Silber, A.; Israeli, Y.; Levi, M.; Keinan, A.; Shapira, O.; Chudi, G.; Golan, A.; Noy, M.; Levkovitch, I.; Assouline, S. Response of 'Hass' avocado trees to irrigation management and root constraint. *Agric. Water Manag.* **2012**, *104*, 95–103. [CrossRef]
- 12. Beyer, C.P.; Cuneo, I.F.; Alvaro, J.E.; Pedreschi, R. Evaluation of aerial and root plant growth behavior, water and nutrient use efficiency and carbohydrate dynamics for Hass avocado grown in a soilless and protected growing system. *Sci. Hortic.* **2021**, 277, 109830. [CrossRef]
- 13. Foster, T.M.; Celton, J.M.; Chagné, D.; Tustin, D.S.; Gardiner, S.E. Two quantitative trait loci, Dw1 and Dw2, are primarily responsible for rootstock-induced dwarfing in apple. *Hortc. Res.* **2015**, *2*, 15001. [CrossRef] [PubMed]
- 14. Fassio, C.; Cautin, R.; Perez-Donoso, A.G.; Castro, M. Comparative branching order and root anatomy of clonal and seed grown avocado trees (*Persea americana Mill.*). *Int. J. Agric. Nat. Resour.* **2020**, *41*, 134–144. [CrossRef]
- 15. Alzugaray, P.; Haase, D.; Rose, R. Efecto del volumen radicular y la tasa de fertilización sobre el comportamiento en terreno de plantas de pino Oregón (*Pseudotsuga menziesii* (Mirb.) Franco producidas con el método 1+1. *Bosques* 2004, 25, 17–33. [CrossRef]
- 16. Castro, M.; Fassio, C.; Darrouy, N. Portainjertos de aguacate en Chile. Hortic. Intern. 2008, 62, 42-46.
- Cabeza, R.A.; Claassen, N. Sistemas radicales de cultivos: Extensión, distribución y crecimiento. Agro Sur 2017, 45, 31–45.
  [CrossRef]
- 18. Holzgreve, H.; Eick, M.; Stöhr, C. Protease activity in the rhizosphere of tomato plants is independent from nitrogen status. In *Root Biology-Growth, Physiology, and Functions*; Ohyama, T., Ed.; IntechOpen: London, UK, 2019; pp. 3–16.
- 19. Lesmes-Vesga, R.A.; Cano, L.M.; Ritenour, M.A.; Sarkhosh, A.; Chaparro, J.X.; Rossi, L. Rhizoboxes as rapid tools for the study of root systems of *Prunus* seedlings. *Plants* **2022**, *11*, 2081. [CrossRef]
- 20. Frolich, E.; Platt, R. Use of the etiolation technique in rooting avocado cuttings. Calif. Avocado Soc. Yearb. 1972, 55, 97–109.
- 21. Bràmoff, M.D.; Magalhães, P.J.; Ram, S.J. Image processing with ImageJ. Biophotonics Intern. 2004, 11, 36–42.
- 22. Seethepalli, A.; Dhakal, K.; Griffiths, M.; Guo, H.; Freschet, G.T.; York, L.M. RhizoVision Explorer: Open-source software for root image analysis and measurement standardization. *AoB Plants* **2021**, *13*, plab056. [CrossRef]
- 23. SAS. SAS® Studio 3.8: Administrator's Guide; SAS Institute Inc.: Cary, NC, USA, 2023; 58p.
- 24. López-Santiago, J.; Nieto-Ángel, R.; Barrientos-Priego, A.F.; Rodríguez-Pérez, E.; Colinas-León, M.T.; Borys, M.W.; González-Andrés, F. Selección de variables morfológicas para la caracterización del tejocote (*Crataegus* spp.). *Rev. Chapingo Ser. Hort.* 2008, 14, 97–111.
- 25. Núñez-Colín, C.A.; Escobedo-López, D. Uso correcto del análisis clúster en la caracterización de germoplasma vegetal. *Agron. Mesoam.* **2011**, 22, 415–427. [CrossRef]
- 26. Hammer, Ø.; Harper, D.A. Past: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* **2001**, *4*, 4.
- 27. Lesmes-Vesga, R.A.; Cano, L.M.; Ritenour, M.A.; Sarkhosh, A.; Chaparro, J.X.; Rossi, L. Variation in the root system architecture of peach×(peach×almond) backcrosses. *Plants* **2023**, *12*, 1874. [CrossRef] [PubMed]
- 28. Cichi, M.; Gheorghiu, N. Aspects regarding variety/rootstock relationship in some species of fruit species. *Ann. Univ. Craiova-Agri. Montanol. Cadastre Ser.* **2022**, *52*, 73–78. [CrossRef]
- 29. Carr, M.K.V. The water relations and irrigation requirements of avocado (*Persea americana* Mill.): A review. *Exp. Agric.* **2013**, 49, 256–278. [CrossRef]
- 30. Lynch, J.P. Harnessing root architecture to address global challenges. Plant J. 2021, 109, 415–431. [CrossRef]
- 31. Atkinson, D.; Wilson, S.A. The root-soil interface and its significance for fruit tree roots of different ages. In *The Soil–Root Interface*; Harley, J.L., Russell, R.S., Eds.; Academic Press: London, UK, 1979; pp. 259–271.

32. Meneses, T.N.; Coelho Filho, M.A.; Santos Filho, H.P.; Santos, L.L.D.A.; Gesteira, A.D.S.; Soares Filho, W.D.S. Rootstocks and planting types on root architecture and vegetative vigor of 'Pera' sweet orange trees. *Rev. Bras. Engn. Agríc. Ambient.* **2020**, 24, 685–693. [CrossRef]

- 33. He, W.; Luo, C.; Wang, Y.; Wen, X.; Wang, Y.; Li, T.; Chen, G.; Zhao, K.; Li, X.; Fan, C. Response strategies of root system architecture to soil environment: A case study of single-species *Cupressus funebris* plantations. *Front. Plant Sci.* **2022**, *13*, 822223. [CrossRef]
- 34. Yang, C.; Fredua-Agyeman, R.; Hwang, S.F.; Gorim, L.Y.; Strelkov, S.E. Genome-wide association studies of root system architecture traits in a broad collection of *Brassica* genotypes. *Front. Plant Sci.* **2024**, *15*, 1389082. [CrossRef]
- 35. Petersson, S.V.; Johansson, A.I.; Kowalczyk, M.; Makoveychuk, A.; Wang, J.Y.; Moritz, T.; Grebe, M.; Benfey, P.N.; Sandberg, G.; Ljung, K. An auxin gradient and maximum in the Arabidopsis root apex shown by high-resolution cell-specific analysis of IAA distribution and synthesis. *Plant Cell* **2009**, *21*, 1659–1668. [CrossRef] [PubMed]
- 36. Barker, R.; Fernandez Garcia, M.N.; Powers, S.J.; Vaughan, S.; Bennett, M.J.; Phillips, A.L.; Thomas, S.G.; Hedden, P. Mapping sites of gibberellin biosynthesis in the *Arabidopsis* root tip. *New Phytol.* **2021**, 229, 1521–1534. [CrossRef] [PubMed]
- 37. Pokhrel, S.; Meyering, B.; Bowman, K.D.; Albrecht, U. Horticultural attributes and root architectures of field-grown 'Valencia' trees grafted on different rootstocks propagated by seed, cuttings, and tissue culture. *HortScience* **2021**, *56*, 163–172. [CrossRef]
- 38. Blois, L.; de Miguel, M.; Bert, P.F.; Ollat, N.; Rubio, B.; Voss-Fels, K.P.; Schmid, J.; Marguerit, E. Dissecting the genetic architecture of root related traits in a grafted wild *Vitis berlandieri* population for grapevine rootstock breeding. *Theor. Appl. Genet.* **2023**, 136, 223. [CrossRef] [PubMed]
- 39. Serrano, A.; Wunsch, A.; Sabety, J.; van Zoeren, J.; Basedow, M.; Miranda Sazo, M.; Fuchs, M.; Khan, A. The comparative root system architecture of declining and non-declining trees in two apple orchards in New York. *Plants* **2023**, *12*, 2644. [CrossRef] [PubMed]
- 40. Yan, W.; Frégeau-Reid, J. Breeding line selection based on multiple traits. Crop Sci. 2008, 48, 417–423. [CrossRef]
- 41. Dutta, C.; Sarma, R.N. Role of root traits and root phenotyping in drought tolerance. *Int. J. Environ. Clim.* **2022**, *12*, 2300–2309. [CrossRef]
- 42. Katuuramu, D.N.; Wechter, W.P.; Washington, M.L.; Horry, M.; Cutulle, M.A.; Jarret, R.L.; Levi, A. Phenotypic diversity for root traits and identification of superior germplasm for root breeding in watermelon. *HortScience* **2020**, *55*, 1272–1279. [CrossRef]

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

Reproduced with permission of copyright owner. Further reproduction prohibited without permission.