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Root Trait Variability in *Coffea canephora* Genotypes and Its Relation to Plant Height and Crop Yield

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Abstract: Coffee breeding based on root traits is important to identify productive genotypes under adverse environmental conditions. This study assessed the diversity of root traits in *Coffea canephora* and its correlation with plant height and crop yield. Undisturbed soil samples were collected down to 60 cm from 43 coffee genotypes, in which one of them was propagated by seed and all others by stem cutting. The roots were washed, scanned, and processed to quantify root length density, root volume, root superficial area, and root diameter. Additionally, plant height and crop yield were also assessed. Root length density ranged from 40 to 1411 mm cm^{-3}, root volume from 6 to 443 mm^3 cm^{-3}, root superficial area from 61 to 1880 mm^2 cm^{-3}, and root diameter from 0.6 to 1.1 mm. Roots were concentrated in the topsoil (0–20 cm) for most genotypes. In deeper depths (30–60 cm), root length density, root volume, and root superficial area were higher in genotypes 14, 25, 31, and 32. Positive correlations were found between root traits and both plant height and crop yield. The results of this work may contribute to the overall cultivation of *C. canephora*, specially for crop breeding in adverse environmental conditions.

Keywords: Conilon coffee; robusta coffee; crop breeding; root morphology; root diversity; root architecture

1. Introduction

Coffee species (*Coffea* spp.) are economically important worldwide. A total of 174 million 60 kg-bags of coffee are annually produced, of which ca. 60% is *Coffea arabica* and ca. 40% *C. canephora* [1]. However, the production of *C. canephora* is threatened by climate change, as it is sensitive to both water deficit and high temperature [2]. The selection of superior genotypes for adverse environmental conditions and the evaluation of diverse genetic pools are therefore essential to ensure its sustainability [3,4].

C. canephora is a self-sterile, diploid and allogamous plant [5,6]. In this view, vegetative propagation is the most common practice, ensuring uniform crop development, high yields, better coffee quality and better maturation patterns [7–9], although it reduces the species genetic diversity. Therefore, exploring the genetic diversity in coffee farms is of utmost importance to achieve crop sustainability [10,11]. Several studies have addressed this topic in order to identify elite genotypes of *C. canephora* [9,12,13]. Current studies have focused on morphological and agronomical traits, leaf anatomy, and nutritional

aspects, aiming not only to identify genotypes with higher yields per se, but also genotypes with higher adaptability and stability in conditions of abiotic stresses [14–17].

In perennial crops, such as *C. canephora*, the root systems are important not only for nutrient and water uptake, but also for ecosystem services such as carbon sequestration, improving soil structure and genetic conservation [18]. Regarding the mechanisms related to coffee adaptation in adverse environments, studies about root performance in coffee during its productive stage are still scarce [19]. Such scarcity is related to the long crop cycle, as coffee is a permanent crop, along with the lack of proper methods for assessing and monitoring coffee roots. According to Ryan et al. [20], such knowledge is unquestionably important, as it has broad and significant implications for the global crop productivity.

Assessments related to root traits have been reviewed for selecting coffee genotypes with better performance under drought conditions [21]. Deeper root systems were associated with drought tolerance in *C. canephora* genotypes due to the larger root dry mass [22]. One of the reviewed studies have assessed both root systems and crop yield, although root assessments were performed in plantlets and yield data were retrieved from previous on-farm data [23]. As root assessments might be cumbersome, they are not widely used in crop breeding programs. Thus, considering that root development and performance are largely influenced by the environment, on-farm studies of the root systems of coffee trees are essential to understand crop performance.

In this study we have characterized the diversity of root traits, plant height and crop yield of 43 *C. canephora* genotypes with a fourfold objective to: (i) assess root distribution (length density, volume, superficial area, and diameter) at the reproductive stage, (ii) analyze the diversity of root traits, (iii) identify potentially promising genotypes to cope with water stress conditions, and (iv) test the correlation between root traits and both crop yield and plant height.

2. Materials and Methods

2.1. Study Site, Soil Characterization and Coffee Genotypes Studied

The study was performed in the municipality of Nova Venécia, northern Espírito Santo State, Brazil (18°39′43′′ S, 40°25′52′′ W and 199 m above sea level). The region's mean annual temperature is 23 °C, with a Aw climate, tropical with hot and humid summer and dry winter, according to Köppen classification [24]. The studied soil was classified as Ferralsol [25], which corresponds to a *Latossolo Amarelo* in the Brazilian soil classification system [26]. Particle size distribution and soil chemical properties are shown in Table 1.

Coffee plantlets with about five pairs of leaves were transplanted in May 2014 with spacing of 3 m × 1 m, which corresponds to a crop density of 3333 coffee trees per hectare. Coffee pruning was performed in order to maintain from three to four orthotropic branches per tree (10,000–12,000 plagiotropic branches per hectare). Farming managements were performed according to the technical guidelines for the crop, aiming to achieve nutritional and phytosanitary needs. Liming and fertilization were performed according to the regional recommendations [27]. The annual rates for N, P_2O_5, and K_2O were 500, 100, and 400 kg ha^{-1}, respectively. In relation to soil micronutrients, a total of 2 kg ha^{-1} Zn, 1 kg ha^{-1} B, 2 kg ha^{-1} Cu, and 10 kg ha^{-1} Mn were applied annually. The area was irrigated by drip irrigation.

A total of 43 coffee genotypes were arranged in randomized blocks with three replicates. Each genotype was considered as a treatment, wherein a group of seven coffee trees of the same genotype constituted an experimental unit, leading to a total of 21 coffee treatments of each genotype and a total number of 903 plants in the experiment. The design was arranged in the double factorial 6 × 43 (six soil depths and 43 coffee genotypes). From the 43 assessed genotypes, 42 were propagated from stem cutting and one genotype was propagated by seed (genotype ID 39), as shown in Table 2.

Table 1. Particle size distribution and soil chemical properties of six soil depths in an irrigated coffee farm located in Nova Venécia, Brazil.

Particle Size Distribution	Soil Depth (cm)					
	0–10	**10–20**	**20–30**	**30–40**	**40–50**	**50–60**
Sand (g kg^{-1})	434	352	188	368	366	376
Silt (g kg^{-1})	86	168	212	32	74	124
Clay (g kg^{-1})	480	480	600	600	560	500
Soil Chemical Properties	**Soil depth (cm)**					
	0–10	**10–20**	**20–30**	**30–40**	**40–50**	**50–60**
K (mg kg^{-1})	110	95	74	57	52	46
S (mg kg^{-1})	15	11	29	15	15	17
Ca (cmol kg^{-1})	3.8	3.4	1.9	1	0.7	0.6
Mg (cmol kg^{-1})	1	0.9	0.4	0.3	0.1	0.1
Al (cmol kg^{-1})	0	0	0.3	0.7	0.8	0.8
H + Al (cmol dm^{-3})	1.6	1.8	2.4	2.9	3.1	3.1
pH-H_2O	6.6	6.5	5.3	4.8	4.8	4.8
SOM (dag kg^{-1})	2.1	1.7	1.1	0.8	0.7	0.5
Fe (mg kg^{-1})	140	138	126	94	88	87
Zn (mg kg^{-1})	10.2	4.5	2.9	1.1	0.6	0.5
Cu (mg kg^{-1})	3.4	4.3	3	1.9	1.2	1
Mn (mg kg^{-1})	207	174	104	46	44	40
B (mg kg^{-1})	0.81	0.83	0.58	0.55	0.56	0.61
Na (mg kg^{-1})	11	37	8	6	5	4
CEC (cmol kg^{-1})	6.73	6.50	4.92	4.37	4.06	3.94

H + Al: potential soil acidity, SOM: soil organic matter, CEC: cation exchange capacity.

Table 2. Identification of 43 genotypes of *C. canephora* cultivated in Nova Venécia, Brazil.

ID	Name	ID	Name	ID	Name
1	Verdim R	16	Pirata	31	Cheique
2	B01	17	Peneirão	32	P2
3	Bicudo	18	Z39	33	Emcapa 02
4	Alecrim	19	Z35	34	Emcapa 153
5	700	20	Z40	35	P1
6	CH1	21	Z29	36	LB1
7	Imbigudinho	22	Z38	37	122
8	AD1	23	Z18	38	Verdim D
9	Graudão HP	24	Z37	39	Sementes
10	Valcir P	25	Z21	40	Emcapa 143
11	Beira Rio 8	26	Z36	41	Ouro negro 1
12	Tardio V	27	Ouro Negro	42	Ouro negro 2
13	AP	28	18	43	Clementino
14	L80	29	Tardio C		
15	Bamburral	30	A1		

Most of the genotypes have not been tested in experimental studies before, as they were selected by regional coffee farmers. Within the *C. canephora* groups, the genotypes assessed are part of the conilon coffee group, which is genetically distinct of the robusta group [28,29]. Genotype 33 belongs to the Emcapa 8111 cultivar, and the genotypes 34 and 39 belong to the Emcapa 8131 cultivar [30], which are late-season cultivars. The genotypes IDs 1, 11, 15, 16, 30 and 43 belong to the Tributum cultivar [8,31], which is recommended for areas under 500 m of altitude in the Brazilian States of Espírito Santo, Southern Bahia, and East Minas Gerais. The genotypes IDs 30 and 35 belong to the Andina cultivar [9], which is recommended for Brazilian states with latitude lower than 22° S, altitude lower than 900 m and minimum air temperature not lower than 8 °C for more than 10 days in a year.

2.2. Root Traits, Plant Height and Crop Yield Assessments

Undisturbed soil samples of about 27 cm^3 were taken with a tubular sampler from six different soil depths (0–10; 10–20; 20–30; 30–40; 40–50; 50–60 cm) in each treatment during February 2018. From each experimental unit, the soil samples were taken from the fourth coffee trees in each one of the three replicates of the experimental plot, which led to a total of 774 soil samples (43 genotypes, three replicates and six soil depths). Each sample was taken from a distance of 30 cm from the coffee stem in relation to the inter-row (Figure 1A), which is a region known for concentrating coffee roots [32]. The reason for sampling the soil down to 60 cm in the soil profile is because this depth best reflects the coffee plant's water status, due to the relation between the vegetative vigor of coffee plants and soil moisture in the root zone [33].

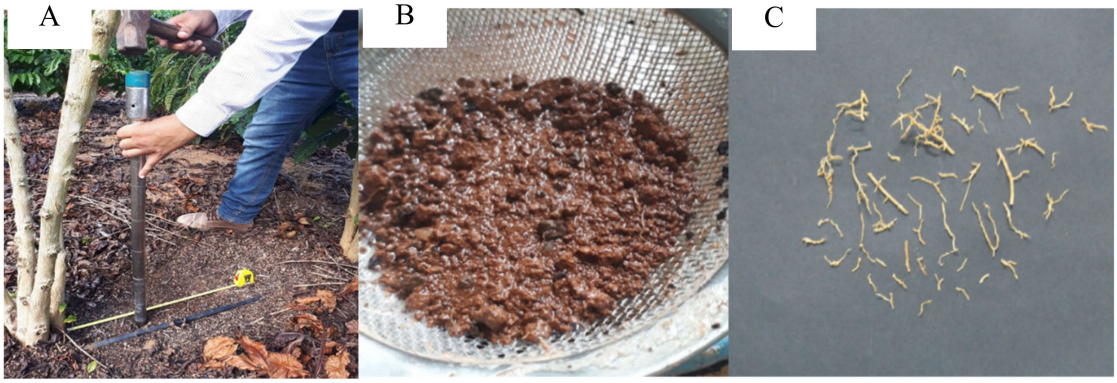

Figure 1. Approach used for sampling the roots of 43 *C. canephora* genotypes, including soil sampling (**A**), washing (**B**) and roots used for scanning (**C**).

The samples were placed in plastic bags, sealed, and stored under −10 °C until further assessments. The samples were thereafter washed (Figure 1B) under running water in a 30 mesh (0.595 mm) sieve in order to separate the roots from the soil (Figure 1C). The few roots with more than 3 mm were excluded from the data set as they were considered outliers. The roots were thereafter scanned with a Nikon 24.1 MP camera model D5200 (Tokyo, Japan), and the images were taken 50 cm above the roots. The resulting images were analyzed with the Safira software version 1.1 [34]. The following traits were assessed: root length density (mm cm^{-3}), root volume (mm^3 cm^{-3}), root superficial area (mm^2 cm^{-3}), and root diameter (mm).

Coffee tree height was assessed with a measuring tape, from base to top. Crop yield was assessed as coffee production per unit of area. It is important to note that the coffee plantlets were transplanted in 2014 and assessments were performed in 2018, wherein the coffee tree height was related to the four-year-old plagiotropic branches. In relation to crop yield, as the coffee plantlets were transplanted in May 2014, the crop yield was assessed annually for three years (2015, 2016, and 2017), and the average crop yield within these three years was used to calculate the correlations with root traits. For both coffee tree height and crop yield, three plants in each experimental unit were assessed in the three replicates, totaling nine plants for each genotype.

2.3. Statistical Analyses

In order to check the analysis of variance assumptions, the data normality was tested by the Shapiro-Wilk test ($p > 0.05$) and the homogeneity of variances by the Bartlett's test ($p > 0.05$) for each soil depth. As the assumptions were not met, data transformation was performed according to the method of Box and Cox [35], using a lambda value (λ) of −1 and the transformed data (Y) equals to $Y^{-1} = 1/Y^1$. After data transformation, the assumptions of normality and homogeneity were met. Thereafter, an analysis of variance for the root traits (variables) versus different coffee genotypes

(treatments), considering the different soil depths as different environments, and a Scott–Knot test ($p < 0.05$) were performed in R version 3.6.1 [36].

In order to group the coffee genotypes according to the assessed traits (root length density, root volume, root superficial area and root diameter), Mahalanobis distance was calculated and used as a measure for dissimilarity. Cluster analysis was then performed by two methods, the Tocher optimization method [37] and the hierarchical unweighted pair group method using arithmetic averages (UPGMA) [38]. The relative contribution of each trait for the diversity within 43 genotypes was analyzed according to Singh [39]. Moreover, Pearson correlation between root traits and both plant height and crop yield were calculated. The analyses were performed in the software Genes [40].

3. Results

3.1. Root Length Density, Volume, Superficial Area, and Root Diameter

Most roots sampled were either short or average-sized (Table 3), with an average mean value for all genotypes and soil depths of 321.13 mm cm^{-3}. The short roots were mostly whitish colored with less than 1 mm diameter. Significant differences were observed between the 43 genotypes of *C. canephora* and the six soil depths for root length density (Table 3), root volume (Table 4), root superficial area (Table 5), and root diameter (Table 6). There was no interaction between coffee genotype and soil depth for root diameter (Table 7), suggesting different root distribution patterns within the assessed coffee genotypes.

Table 3. Average root length density of 43 *C. canephora* genotypes in six soil depths.

	Root Length Density (mm cm^{-3})					
ID	**0–10 cm**	**10–20 cm**	**20–30 cm**	**30–40 cm**	**40–50 cm**	**50–60 cm**
1	598.67 Ab	252.46 Bc	408.23 Aa	191.19 Bb	191.80 Bb	212.70 Bb
2	458.86 Ac	327.43 Ac	108.47 Bc	69.29 Bc	76.75 Bc	118.53 Bc
3	958.89 Aa	753.29 Aa	392.22 Ba	253.41 Bb	152.59 Cc	154.06 Cc
4	1223.22 Aa	524.14 Bb	305.28 Cb	237.74 Cb	167.39 Cc	143.97 Cc
5	1151.99 Aa	361.13 Bb	246.98 Bb	249.24 Bb	55.82 Cc	53.53 Cd
6	1038.86 Aa	269.18 Bc	213.55 Bb	206.88 Bb	200.18 Bb	134.21 Bc
7	684.90 Ab	222.33 Bc	244.23 Bb	189.09 Bb	140.92 Bc	137.38 Bc
8	577.47 Ab	269.98 Bc	200.29 Bc	297.53 Bb	375.66 Ba	320.27 Ba
9	1041.41 Aa	385.07 Bb	143.48 Cc	219.99 Cb	159.67 Cc	92.93 Cd
10	886.69 Aa	332.29 Bc	115.42 Cc	101.29 Cc	156.29 Cc	120.78 Cd
11	643.51 Ab	287.73 Bc	169.73 Cc	131.66 Cc	126.92 Cc	107.75 Cc
12	808.37 Aa	272.60 Bc	126.21 Cc	105.56 Cc	84.38 Cc	133.20 Cc
13	1038.56 Aa	533.22 Bb	331.69 Cb	276.04 Cb	150.67 Dc	85.54 Dd
14	954.66 Aa	902.55 Aa	654.78 Aa	610.63 Aa	410.51 Ba	419.35 Ba
15	844.12 Aa	295.78 Bc	359.16 Ba	209.02 Bb	210.17 Bb	226.18 Bb
16	545.08 Ab	208.68 Bc	187.97 Bc	150.98 Bc	158.23 Bc	156.50 Bc
17	924.88 Aa	532.41 Bb	155.40 Cc	108.92 Cc	98.96 Cc	125.60 Cc
18	913.18 Aa	333.6 Bc	224.36 Bb	144.61 Cc	150.83 Cc	135.34 Cc
19	894.91 Aa	664.16 Aa	429.52 Ba	212.14 Cb	179.70 Cc	200.67 Cb
20	439.05 Ac	176.23 Bd	94.50 Bc	222.94 Bb	135.48 Bc	159.05 Bb
21	945.22 Aa	364.88 Bb	121.98 Cc	165.99 Cc	142.98 Cc	101.70 Cd
22	532.43 Ab	317.34 Bc	151.95 Cc	100.55 Cc	125.68 Cc	99.04 Cd
23	347.84 Ac	205.32 Bc	173.45 Bc	154.13 Cc	107.64 Cc	78.54 Cd
24	1107.84 Aa	459.30 Bb	280.22 Bb	134.91 Cc	155.38 Cc	155.43 Cc
25	1127.29 Aa	737.96 Ba	527.82 Ca	255.99 Cb	332.09 Ca	295.84 Ca
26	850.98 Aa	339.77 Bc	256.65 Bb	56.94 Cc	103.93 Cc	138.18 Cc
27	623.14 Ab	190.75 Bd	138.87 Bc	102.75 Bc	69.45 Cc	39.56 Cd

Table 3. *Cont.*

	Root Length Density (mm cm^{-3})					
ID	**0–10 cm**	**10–20 cm**	**20–30 cm**	**30–40 cm**	**40–50 cm**	**50–60 cm**
28	528.74 Ab	178.76 Bd	156.95 Bc	142.98 Bc	142.76 Bc	129.70 Bc
29	441.14 Ac	257.89 Bc	144.72 Cc	136.42 Cc	97.82 Dc	56.67 Dd
30	870.18 Aa	407.97 Bb	317.66 Bb	232.64 Cb	224.72 Cb	167.00 Cb
31	334.02 Ac	119.36 Bd	206.52 Ab	132.78 Bc	109.45 Bc	230.45 Ab
32	1411.17	339.12 Bc	314.49 Bb	225.44 Bb	182.07 Cb	141.21 Cc
33	493.82 Ab	143.53 Bd	118.96 Bc	123.64 Bc	109.14 Bc	70.62 Bd
34	799.76 Aa	392.70 Bc	160.75 Cc	133.34 Cc	140.51 Cc	139.59 Cc
35	935.75 Aa	414.44 Bb	234.07 Cb	221.27 Cb	187.26 Cb	192.63 Cb
36	710.54 Ab	405.30 Bb	235.65 Cb	192.58 Cb	137.05 Cc	184.30 Cb
37	941.13 Aa	207.82 Bc	157.60 Bc	143.09 Bc	94.33 Bc	111.28 Bc
38	1138.42 Aa	440.56 Bb	217.78 Cb	180.90 Cc	295.66 Ba	183.65 Cb
39	651.18 Ab	255.66 Bc	255.83 Bb	161.77 Cc	124.25 Cc	141.16 Cc
40	1113.61 Aa	709.92 Ba	435.70 Cb	275.21 Cb	256.85 Cb	256.94 Cb
41	1035.80 Aa	504.23 Bb	316.17 Cb	294.56 Cb	151.05 Dc	146.91 Dc
42	537.34 Aa	104.66 Bd	79.05 Bc	134.27 Bc	70.82 Bc	72.66 Bd
43	1013.91 Aa	397.49 Bb	283.02 Bb	205.45 Cb	209.41 Cb	118.23 Cc

Different lowercase letters in the same column and different uppercase letters in the same row statistically differ from each other according to the Scott-Knott test ($p < 0.05$).

Table 4. Average root volume of 43 *C. canephora* genotypes in six soil depths.

	Root Volume (mm^3 cm^{-3})					
ID	**0–10 cm**	**10–20 cm**	**20–30 cm**	**30–40 cm**	**40–50 cm**	**50–60 cm**
1	139.45 Ac	43.70 Bd	92.78 Aa	53.01 Ba	44.41 Bb	35.71 Ba
2	97.48 Ad	45.72 Bd	30.75 Cc	10.14 Cb	20.65 Cc	51.90 Ba
3	181.41 Ab	177.47 Aa	137.26 Ac	41.70 Ba	34.21 Bb	40.39 Ba
4	254.84 Ab	192.45 Aa	89.94 Bb	52.88 Ba	62.25 Bb	67.22 Ba
5	206.97 Ab	63.97 Aa	59.62 Bb	42.47 Ba	6.40 Dd	18.10 Cb
6	215.30 Ab	50.59 Aa	48.24 Bb	57.00 Ba	47.22 Bb	36.14 Ba
7	142.79 Ac	39.16 Bd	56.78 Bb	39.57 Ba	24.73 Bc	28.36 Bb
8	118.40 Ac	72.91 Bc	45.07 Bc	56.52 Ba	122.75	74.61 Ba
9	208.03 Ab	94.18 Bb	66.22 Bb	39.61 Ba	41.64 Bb	65.60 Ba
10	132.91 Ac	77.84 Bc	40.06 Bc	17.24 Cb	43.60 Bb	21.14 Cc
11	146.55 Ac	57.12 Bc	42.72 Bc	21.12 Bb	24.96 Bc	20.76 Bb
12	158.97 Ac	93.18 Bb	29.87 Bc	14.35 Bb	14.67 Bd	52.17 Ca
13	184.40 Ab	82.86 Bb	72.23 Cb	60.58 Ca	23.47 Dc	30.66 Db
14	170.40 Ab	169.08 Aa	128.91 Aa	138.04 Aa	112.82 Aa	62.72 Ba
15	189.33 Ab	109.41 Ab	95.05 Aa	45.47 Ba	53.83 Bb	69.09 Ba
16	153.04 Ac	55.08 Bc	48.52 Bb	32.06 Ba	35.29 Bb	42.49 Ba
17	207.37 Ab	144.26 Aa	51.33 Bb	25.83 Cb	30.80 Cc	25.06 Cb
18	131.88 Ac	61.09 Bc	46.63 Cc	18.28 Cb	18.25 Cc	23.70 Cb
19	209.27 Cb	132.40 Ab	66.20 Bb	40.53 Ba	31.33 Bc	34.53 Ba
20	77.74 Ad	34.06 Bd	17.49 Bc	45.50 Aa	25.72 Bc	63.76 Aa
21	243.65 Ab	94.60 Bb	49.29 Cc	30.09 Cb	36.35 Cc	32.02 Cb
22	104.91 Ad	50.30 Bc	27.48 Cc	19.60 Cb	16.10 Cc	11.80 Cc
23	67.69 Ad	84.44 Ab	28.47 Bc	50.12 Aa	48.20 Ab	11.86 Bc
24	161.48 Ac	101.61 Ab	66.99 Bb	25.19 Bb	33.81 Bb	38.65 Ba
25	198.28 Ab	146.62 Aa	88.86 Ba	46.28 Ba	71.60 Ba	70.19 Ba
26	194.94 Ab	87.04 Bc	71.30 Bb	8.95 Cb	21.27 Cc	63.81 Ba
27	127.98 Ac	39.23 Bd	45.38 Bc	17.21 Cb	10.92 Cd	11.18 Cc

Table 4. *Cont.*

	Root Volume (mm^3 cm^{-3})					
ID	**0–10 cm**	**10–20 cm**	**20–30 cm**	**30–40 cm**	**40–50 cm**	**50–60 cm**
28	128.08 Ac	40.12 Bd	34.61 Bc	34.16 Ba	25.02 Bc	37.52 Ba
29	69.87 Ad	52.54 Ac	29.72 Bc	33.77 Ba	40.24 Bc	7.67 Cc
30	215.35 Ab	97.90 Bb	72.05 Bb	35.62 Ca	55.37 Bb	36.50 Ca
31	86.23 Ad	28.13 Bd	32.45 Bc	48.67 Ba	30.53 Bc	68.58 Aa
32	443.29 Aa	70.35 Bc	69.37 Bb	70.23 Ba	45.69 Bb	28.38 Cb
33	133.90 Ac	32.03 Bd	22.45 Bc	26.68 Bb	24.01 Bc	12.06 Bc
34	148.04 Ac	92.20 Bc	34.17 Bc	64.90 Ba	36.05 Bc	49.08 Ba
35	219.62 Ab	65.81 Bc	56.99 Bb	40.46 Ba	29.57 Bc	33.82 Ba
36	158.61 Ac	101.46 Ab	41.31 Bc	49.75 Ba	32.27 Bc	49.70 Ba
37	208.15 Ab	39.48 Bd	19.81 Bc	30.13 Ba	25.23 Bc	18.64 Bb
38	210.20 Ab	118.58 Bb	35.63 Cc	35.34 Ca	43.58 Cb	58.47 Ca
39	162.86 Ac	54.46 Bc	41.93 Bc	33.50 Ba	25.03 Bc	50.54 Ba
40	220.27 Ab	125.44 Ab	98.37 Bb	82.77 Ba	48.32 Bb	45.25 Ba
41	251.32 Ab	103.31 Bb	64.03 Cb	56.82 Ca	39.69 Cb	34.25 Ca
42	112.76 Ac	24.20 Bd	17.52 Bc	11.23 Bb	8.26 Bd	11.15 Bc
43	282.15 Ab	165.73 Aa	50.08 Bc	44.80 Ba	55.55 Bb	25.72 Bb

Different lowercase letters in the same column and different uppercase letters in the same row statistically differ from each other according to the Scott-Knott test ($p < 0.05$).

Table 5. Average root superficial area of 43 *C. canephora* genotypes in six soil depths.

	Root Superficial Area (mm^2 cm^{-3})					
ID	**0–10 cm**	**10–20 cm**	**20–30 cm**	**30–40 cm**	**40–50 cm**	**50–60 cm**
1	1119.52 Aa	340.73 Bc	479.65 Bb	311.03 Bb	297.10 Bb	286.74 Bb
2	671.87 Ac	411.53 Bc	184.47 Cc	89.26 Cc	122.37 Cc	246.17 Cb
3	1342.31 Aa	1209.47 Aa	668.95 Ba	342.59 Cb	228.01 Cc	258.40 Cb
4	1761.73 Aa	994.44 Ba	524.58 Cb	380.70 Cb	293.16 Cb	289.08 Cb
5	1571.17 Aa	497.98 Ac	420.11 Bb	335.19 Bb	65.38 Cc	100.15 Cc
6	1518.57 Aa	382.24 Bc	326.79 Bc	334.58 Bb	317.67 Bb	218.98 Bb
7	985.56 Ab	305.62 Bd	373.46 Bb	276.44 Bb	196.48 Bc	197.99 Bc
8	852.72 Ab	441.67 Bc	307.49 Bc	427.98 Bb	618.60 Aa	505.74 Ba
9	1511.61 Aa	599.40 Bb	240.40 Cc	310.70 Cb	249.68 Cb	189.45 Cc
10	1138.91 Aa	519.54 Bc	212.45 Cc	141.90 Cc	257.78 Bb	165.41 Cc
11	981.87 Ab	410.42 Bc	316.26 Bc	175.54 Cc	186.52 Cc	155.21 Cc
12	1161.38 Aa	462.23 Bc	198.85 Cc	131.95 Cc	117.55 Cc	252.56 Cb
13	1436.98 Aa	702.89 Bb	478.14 Cb	404.17 Cb	197.90 Dc	153.64 Dc
14	1324.95 Aa	1279.19 Aa	953.64 Aa	795.00 Aa	661.81 Ba	541.55 Ba
15	1284.13 Aa	567.63 Bb	597.01 Ba	318.89 Cb	342.14 Cb	402.29 Ca
16	912.52 Ab	340.19 Bc	291.56 Bc	225.26 Bc	239.60 Bb	256.00 Bb
17	1355.46 Aa	853.76 Ba	274.34 Cc	171.25 Cc	137.15 Cc	186.58 Cc
18	1149.74 Aa	459.71 Bc	326.75 Bc	174.73 Cc	179.76 Cc	188.08 Cc
19	1344.29 Aa	949.55 Aa	559.85 Ba	300.47 Cb	247.82 Cc	274.31 Cb
20	596.36 Ac	255.65 Bd	134.17 Bc	321.61 Bb	193.16 Bc	311.98 Bb
21	1526.81 Aa	584.46 Bb	189.16 Cc	230.20 Cc	230.56 Cc	175.50 Cc
22	735.33 Ab	418.15 Bc	208.48 Cc	146.86 Cc	152.82 Cc	117.64 Cc
23	503.41 Ac	391.36 Ac	234.10 Bc	224.70 Bc	204.52 Bc	102.76 Bc
24	1418.18 Aa	671.54 Bb	415.63 Cb	191.43 Cc	233.44 Cb	249.29 Cb
25	1555.80 Aa	1054.59 Aa	713.45 Ba	355.22 Bb	481.37 Ba	463.25 Ba
26	1328.02 Aa	548.88 Bc	429.96 Bb	75.08 Cc	155.60 Cc	265.21 Bb
27	925.87 Ab	280.76 Bd	230.19 Bc	139.76 Bc	92.45 Cc	61.10 Cc
28	817.34 Ab	276.28 Bd	239.70 Bc	227.28 Bc	214.10 Bc	210.39 Bb
29	578.51 Ac	382.57 Ac	215.90 Bc	212.17 Bc	189.10 Bc	70.72 Cc
30	1384.86 Aa	641.57 Bb	567.97 Ba	305.39 Cb	349.21 Cb	250.36 Cb

Table 5. *Cont.*

	Root Superficial Area (mm^2 cm^{-3})					
ID	**0–10 cm**	**10–20 cm**	**20–30 cm**	**30–40 cm**	**40–50 cm**	**50–60 cm**
31	542.14 Aa	144.26 Bd	213.46 Bc	238.77 Bb	180.93 Bc	374.49 Aa
32	1879.78 Aa	486.59 Bc	461.56 Bb	359.13 Bb	293.41 Cb	202.88 Cc
33	779.34 Ab	210.92 Bd	169.13 Bc	185.81 Bc	166.28 Bc	97.03 Bc
34	1136.64 Aa	328.50 Bc	243.13 Cc	279.35 Cb	220.12 Cc	254.56 Cb
35	1407.29 Aa	540.49 Bb	371.29 Bb	314.73 Bb	243.98 Bb	268.54 Bb
36	1060.10 Aa	622.37 Bb	329.52 Cc	306.99 Cb	218.98 Cc	298.66 Cb
37	1418.26 Aa	295.47 Bd	200.46 Bc	216.26 Bc	158.65 Bc	128.33 Bc
38	1589.96 Aa	720.32 Bb	285.75 Cc	285.86 Cb	264.60 Cb	320.76 Cb
39	1041.81 Ab	384.43 Bc	343.62 Bc	239.21 Bb	181.00 Bc	264.06 Bb
40	1606.03 Aa	962.71 Ba	631.57 Cc	397.19 Cb	378.15 Cb	352.45 Ca
41	1614.90 Aa	750.93 Bb	528.71 Cb	417.36 Cb	280.94 Cb	232.87 Cb
42	804.68 Ab	163.67 Bd	121.59 Bc	89.85 Bc	101.34 Bc	95.77 Bc
43	1694.49 Aa	787.90 Bb	392.30 Cb	308.25 Cb	328.07 Cb	177.76 Cc

Different lowercase letters in the same column and different uppercase letters in the same row statistically differ from each other according to the Scott-Knott test ($p < 0.05$).

Table 6. Average root diameter of 43 *C. canephora* genotypes in six soil depths.

	Root Diameter (mm)					
ID	**0–10 cm**	**10–20 cm**	**20–30 cm**	**30–40 cm**	**40–50 cm**	**50–60 cm**
1	0.79 Aa	0.73 Ab	0.77 Aa	0.73 Aa	0.71 Aa	0.74 Aa
2	0.88 Aa	0.73 Ab	0.71 Ab	0.66 Aa	0.68 Aa	0.7 Aa
3	0.94 Aa	1.01 Aa	0.92 Aa	0.75 Ba	0.73 Ba	0.74 Ba
4	0.95 Aa	0.84 Aa	0.88 Aa	0.79 Aa	0.79 Aa	0.79 Aa
5	0.82 Aa	0.88 Aa	0.85 Aa	0.82 Aa	0.62 Ba	0.66 Ba
6	1.01 Aa	0.79 Bb	0.77 Ba	0.76 Ba	0.73 Ba	0.71 Ba
7	0.76 Aa	0.79 Ab	0.64 Ab	0.77 Aa	0.75 Aa	0.74 Aa
8	0.9 Aa	0.87 Aa	0.75 Ab	0.79 Aa	0.85 Aa	0.84 Aa
9	0.99 Aa	0.97 Aa	0.78 Ba	0.77 Ba	0.75 Ba	0.74 Ba
10	0.88 Aa	0.81 Ab	0.79 Aa	0.74 Aa	0.67 Aa	0.64 Aa
11	0.92 Aa	0.83 Aa	0.83 Aa	0.70 Ba	0.70 Ba	0.67 Ba
12	1.01 Aa	0.92 Aa	0.75 Bb	0.67 Ba	0.65 Ba	0.87 Aa
13	0.92 Aa	0.79 Ab	0.74 Ab	0.73 Aa	0.70 Aa	0.7 Aa
14	0.95 Aa	0.93 Aa	0.88 Aa	0.84 Aa	0.81 Aa	0.77 Aa
15	0.95 Aa	0.86 Aa	0.84 Aa	0.81 Aa	0.79 Aa	0.80 Aa
16	1.07 Aa	0.79 Bb	0.77 Ba	0.73 Ba	0.75 Ba	0.76 Ba
17	0.99 Aa	0.90 Aa	0.96 Aa	0.74 Ba	0.66 Ba	0.79 Ba
18	0.92 Aa	0.75 Bb	0.69 Bb	0.62 Ba	0.65 Ba	0.66 Ba
19	0.99 Aa	0.70 Bb	0.75 Bb	0.73 Ba	0.70 Ba	0.75 Ba
20	0.87 Aa	0.78 Ab	0.74 Ab	0.81 Aa	0.79 Aa	0.76 Aa
21	1.04 Aa	0.83 Ba	0.79 Ba	0.80 Ba	0.78 Ba	0.73 Ba
22	0.93 Aa	0.81 Ab	0.79 Aa	0.66 Ba	0.66 Ba	0.64 Ba
23	0.88 Aa	0.73 Bb	0.65 Bb	0.67 Ba	0.66 Ba	0.64 Ba
24	1.02 Aa	0.86 Ba	0.78 Ba	0.70 Ba	0.75 Ba	0.73 Ba
25	0.92 Aa	0.88 Aa	0.79 Aa	0.77 Aa	0.78 Aa	0.80 Aa
26	0.93 Aa	0.83 Aa	0.81 Aa	0.72 Aa	0.77 Aa	0.84 Aa
27	0.91 Aa	0.75 Ab	0.79 Aa	0.66 Ba	0.62 Ba	0.64 Ba
28	0.88 Aa	0.70 Bb	0.66 Bb	0.66 Ba	0.65 Ba	0.65 Ba
29	0.83 Aa	0.75 Ab	0.73 Ab	0.71 Aa	0.69 Aa	0.66 Aa
30	0.97 Aa	0.71 Bb	0.69 Bb	0.71 Ba	0.73 Ba	0.70 Ba
31	0.92 Aa	0.75 Ab	0.84 Aa	0.77 Aa	0.75 Aa	0.82 Aa

Table 6. *Cont.*

	Root Diameter (mm)					
ID	**0–10 cm**	**10–20 cm**	**20–30 cm**	**30–40 cm**	**40–50 cm**	**50–60 cm**
32	0.94 Aa	0.79 Bb	0.97 Aa	0.97 Aa	0.91 Aa	0.76 Ba
33	1,00 Aa	0.83 Ba	0.79 Ba	0.79 Ba	0.70 Ba	0.69 Ba
34	0.91 Aa	0.76 Ab	0.84 Aa	0.83 Aa	0.79 Aa	0.81 Aa
35	0.97 Aa	0.85 Aa	0.86 Aa	0.83 Aa	0.75 Aa	0.79 Aa
36	0.94 Aa	0.82 Aa	0.78 Aa	0.78 Aa	0.75 Aa	0.77 Aa
37	1.00 Aa	0.71 Bb	0.62 Bb	0.69 Ba	0.65 Ba	0.68 Ba
38	0.85 Aa	0.91 Aa	0.84 Aa	0.79 Aa	0.84 Aa	0.88 Aa
39	0.97 Aa	0.80 Bb	0.79 Ba	0.70 Ba	0.68 Ba	0.75 Ba
40	0.97 Aa	0.92 Aa	0.59 Bb	0.71 Ba	0.70 Ba	0.70 Ba
41	0.92 Aa	0.83 Aa	0.8 Aa	0.75 Aa	0.71 Aa	0.70 Aa
42	0.79 Aa	0.70 Ab	0.66 Ab	0.66 Aa	0.64 Aa	0.65 Aa
43	1.00 Aa	0.92 Aa	0.79 Ba	0.75 Ba	0.80 Ba	0.73 Ba

Different lowercase letters in the same column and different uppercase letters in the same row statistically differ from each other according to the Scott-Knott test ($p < 0.05$).

Table 7. Analysis of variance of four root traits (root length density, root volume, root superficial area, and root diameter) for 43 genotypes of *C. canephora* grown in Nova Venécia, Brazil.

		Mean Square			
Trait	**Mean value**	**Genotype**	**Soil depth**	**Genotype x soil depth**	**Residue**
Root length density (mm cm^{-3})	321.13	82.08 **	2599.75 **	10.79 **	5.03
Root volume (mm^3 cm^{-3})	71.68	14.87 **	350.12 **	2.76 **	1.14
Root superficial area (mm^2 cm^{-3})	477.79	174.31 **	5320.79 **	23.04 **	10.60
Root diameter (mm)	0.79	0.06 **	1.02 **	0.01	0.02

The Mean Square only applies to the four columns in the right. ** Significant at $p < 0.05$ by the F test.

Most coffee roots were concentrated in the 0–10 and 10–20 cm depths. This layer (0–20 cm) concentrated 61.56 % of total root length density (Table 3), 61.57% of total root volume (Table 4), and 61.10% of total root superficial area (Table 5). Results from deeper depths were more evenly distributed.

Three out of the four root assessments, root length density (Table 3), root volume (Table 4), and root superficial area (Table 5), grouped the studied genotypes in four distinct groups (as the lowercase letters ranged from *a* to *d*) based on data from all depths combined. However, considering the assessed soil depths, the 20–30 and 30–40 cm layers formed at least three distinct groups for all four root assessments (Tables 3–6).

3.2. Cluster Analyses

Coffee genotypes were grouped into four different groups (Figure 2), considering a cut-off point of 38% of dissimilarity in the dendogram, as recommended by Mojena [31]. In order to better understand the five different groups formed, the mean averages for root length density, root superficial area, and root volume were calculated for each group (Figure 3). Group I was composed of genotypes ID 1, 2, 5, 6, 7, 9, 10, 11, 12, 16, 17, 18, 20, 21, 22, 23, 26, 27, 28, 29, 31, 33, 34, 36, 37, 39, and 42. This group differed from the others with 39% of accuracy. Root length density (Figure 3A), root volume (Figure 3B) and root superficial area (Figure 3C) were lower in this group than all others. Furthermore, it allocated genotypes ID 21 and 34, which showed the highest similarity between two genotypes. Within group I, the genotype ID 31 concentrated 20.35% of total root length density (Figure 3B), 23.28% of root volume (Figure 3D) and 22.11% of root superficial (Figure 3F) area in the 50–60 cm soil depth.

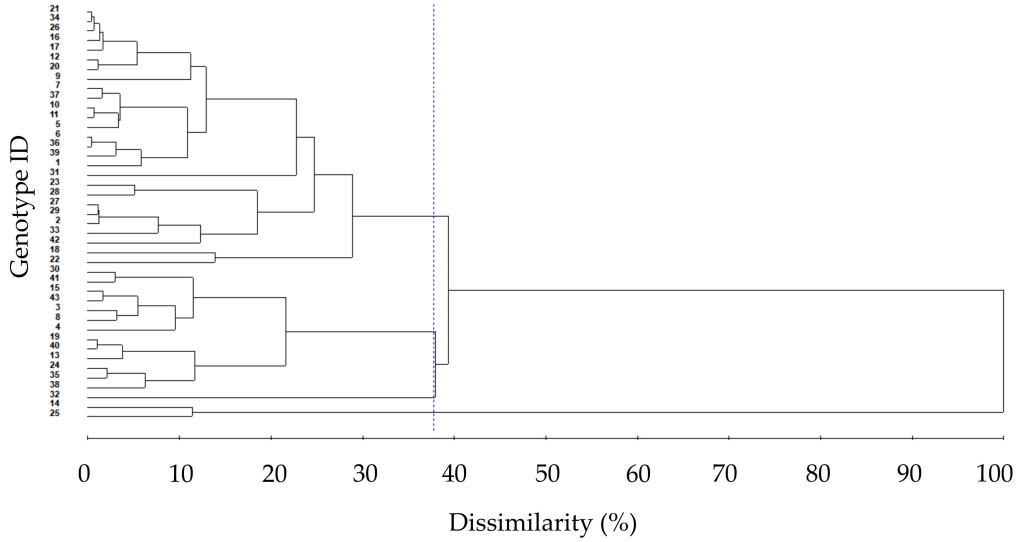

Figure 2. Dissimilarity within 43 *C. canephora* genotypes using the Mahalanobis distance and the unweighted pair group method with arithmetic mean (UPGMA), considering four root traits (root length density, root volume, root superficial area, and root diameter) and six soil depths (0–10, 10–20, 20–30, 30–40, 40–50, and 50–60 cm).

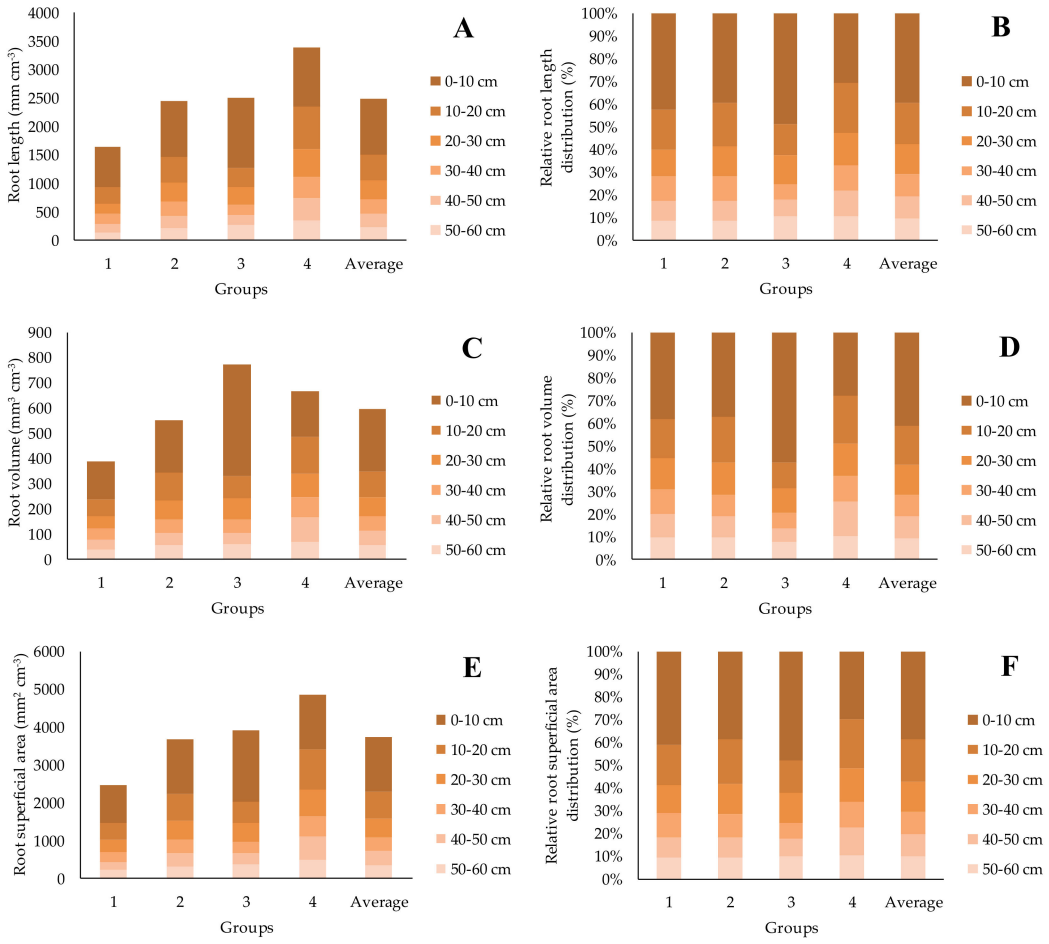

Figure 3. Mean values (**A**,**C**,**E**) and relative distribution within each group (**B**,**D**,**F**) for root length density (**A**,**B**), root volume (**C**,**D**), and root superficial area (**E**,**F**) in six soil depths (0–10, 10–20, 20–30, 30–40, 40–50, and 50–60 cm).

Group II was composed of genotypes ID 3, 4, 8, 13, 15, 19, 24, 30, 35, 38, 40, 41, and 43. Group III was composed only of genotype ID 32, which had the highest root volume (Figure 3C), mainly concentrated in the topsoil (Figure 3D). The root traits of group IV (comprising genotypes ID 14 and 25) were more evenly distributed within the six soil depths than all other groups (Figure 3). Group IV differed from all other groups, with a dissimilarity to all other genotypes over 99%. This group had the highest root length density (Figure 3A,B) and root superficial area (Figure 3E,F).

It is important to note the results for the seed-propagated genotype (genotype 39) in relation to all others, propagated by stem cutting. This genotype is within group I, the group with the highest number of different coffee genotypes (Figure 2). Intermediate values were found for the root traits in the seed-propagated genotype, in which other genotypes (propagated by stem cutting) had higher, similar, and lower values.

The Tocher clustering method formed ten distinct groups (Table 8). The higher number of groups indicates a broad genetic diversity between coffee genotypes, as the method seeks to minimize intragroup distance and maximize intergroup distance. Most (25 out of 43) of the genotypes were located within groups I and II. Group III was composed of five coffee genotypes (ID 02, 27, 29, 33, and 42). Groups IV, V, VI, VII, VIII, and IX were composed of two genotypes each, and group X was composed of only one coffee genotype (31). Root superficial area, root length density, root volume and root diameter had a relative contribution for the diversity within genotypes of 43.1%, 33.9%, and 6.5%, respectively.

Table 8. Comparison clusters by each clustering method using the Mahalanobis distance (UPGMA and Tocher methods) of 43 genotypes of *C. canephora* considering four root traits (root length density, root volume, root superficial area, and root volume) and six soil depths (0–10, 10–20, 20–30, 30–40, 40–50, and 50–60 cm).

Groups (Tocher)	Genotype ID	Corresponding Group (UPGMA)
I	21, 34, 26, 16, 17, 12, 39, 36, 20, 11, 10, 06, 05, 07, 24 *, 37	
III	27, 29, 02, 33, 42	
VI	23, 28	I
VIII	18, 22	
X	31	
II	19, 40, 13, 01 *, 30, 41, 03, 08, 43	
IV	04, 15	II
V	35, 38	
IX	09 *, 32	III
VII	14, 25	IV

* Genotype 24 was placed in group II by UPGMA, genotypes 1 and 9 were both placed in group I by UPGMA.

3.3. Root Traits, Plant Height and Crop Yield Correlations

There were significant positive correlations between root traits and plant height, as well as root traits and crop yield (Figure 4), with different levels of significance. However, such correlations were weak ($r < 0.3$) or moderate ($0.3 < r < 0.6$). Within root traits, the highest correlation was found between root length density and superficial area (0.99), followed by root superficial area and root volume (0.96). The lowest correlations were found for root diameter and plant height (0.25), rot diameter and crop yield (0.25), and plant height and crop yield (0.22).

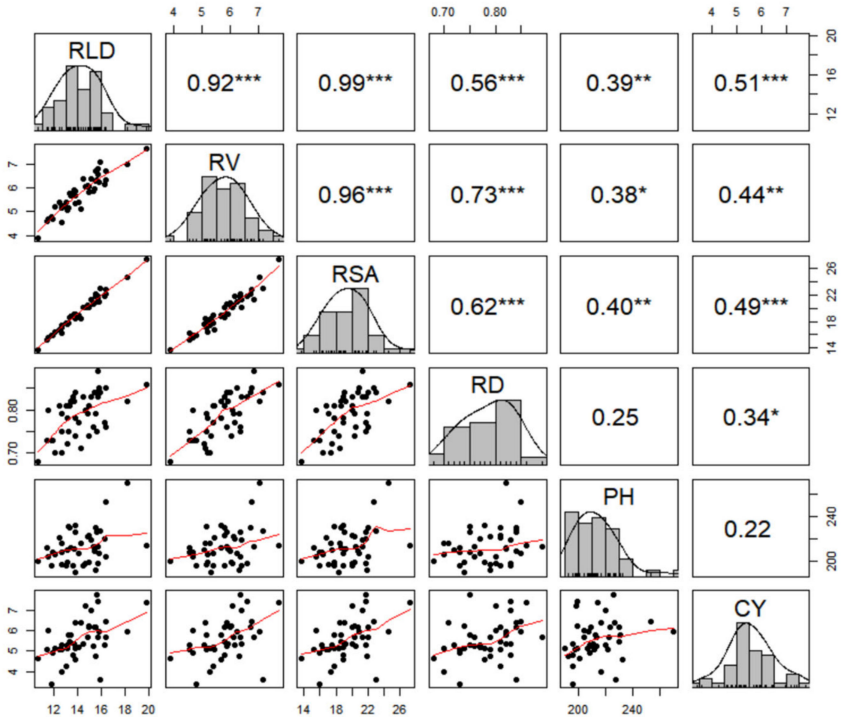

Figure 4. Correlation root length density, root volume, root superficial area, root diameter, plant height, and crop yield from 43 *C. canephora*. * Significant at $p < 0.1$, ** significant at $p < 0.05$, *** significant at $p < 0.01$. The red lines indicate the trend lines between variables. RLD: root length density, RV: root volume, RSA root superficial area, RD: root diameter, PH: plant height, CY: crop yield.

4. Discussion

4.1. Root Length Density, Volume, Superficial Area, and Root Diameter

Short or average-sized (Table 3) roots are considered absorbing roots [41]. In ideal conditions for root development, coffee genotypes grow efficiently with absorbing roots concentrated within the topsoil (down to 30 cm deep). However, under adverse conditions, such as severe drought, coffee genotypes with deeper roots may access more water and thus be better adapted to such conditions [42]. Studies from Ronchi et al. [43] and Isaac et al. [44] indicated that the root system is directly related to crop adaptation and crop yield.

The root distribution patterns, which were concentrated in the topsoil, are in accordance with the work of Partelli et al. [45], who found about 60% of coffee roots within soil depths of 0–20 and 25–50 cm. Furthermore, in the subsoil, the 20–30 and 30–40 cm layers formed at least three distinct groups for all four root assessments (Tables 3–6). Similarly, Defrenet et al. [46] assessed root biomass and necromass in a coffee agroforestry system and found that most roots were fine and located in the topsoil. Crop breeding for potentially promising coffee genotypes under water stress should thus be performed in soil depths of 40 cm or deeper.

It is also important to note that deeper soil depths generally imply higher soil resistance to root penetration due to higher soil compaction [47], although coffee roots may penetrate down to four meters in the soil profile [46]. Another possible factor related to higher resistance to root penetration in the soil subsurface is the higher aluminum content, which is responsible for soil acidity [48]. The above-mentioned relationships are in accordance with this study results, as the 20–40 cm layer presented higher Al content (Table 1) and lower root abundancy (Tables 3–6) than the topsoil.

According to Rao et al. [49], crop adaptation for infertile soils may be achieved from two options: either the growing medium may be changed or the plant genotype may be bred. Considering the current concerns about climate change and soil degradation, both options should be integrated [19].

According to Gould et al. [50], architectural root traits, as the ones studied in this work, positively impact soil structure, forming effective hydraulic pathways in the soil, and promoting a better environment for water and nutrients uptake.

4.2. Cluster Analyses

The cluster analysis suggests that group IV comprises promising genotypes to cope with water stress conditions, as their roots were more evenly distributed within soil depths, and it had consequently higher root abundance in deeper layers in comparison with groups I, II and III. The deeper root abundance in *C. canephora* genotypes are commonly related to drought tolerance due to the larger root dry mass [22]. Thus, genotypes from group IV (ID 14 and 25) may be a promising alternative for *C. canephora* breeding due to their root system distribution.

The intermediate root traits values for the seed-propagated genotype (39), suggest that the root system development is more related to the plant genetic than to the propagation method, as other genotypes (propagated by stem cutting) had higher, similar, and lower values. Partelli et al. [7] found similar results, with no difference in the root system distribution of *C. canephora* plants propagated by seeds in relation to plants propagated by stem cutting.

According to Hair Jr. et al. [51], cluster analyses may be divided in hierarchical and non-hierarchical methods. Ideally, studies should consider both approaches and thereafter ensure a more detailed result. Thus, apart from the UPGMA clustering (hierarchical method), this study also tested the Tocher optimization method (Table 8) by using the Mahalanobis distance as the genetic dissimilarity between genotypes. The Tocher method had been successfully used in *C. canephora* in previous studies [13,14,52] to identify promising genotypes with greater genetic variability.

Both clustering methods led to somehow similar results (Table 8). Genotypes ID 14, 25, and 32 were grouped separately in both methods, which strengthens the suggestion that they are promising alternatives for *C. canephora* breeding due to their distinct root system distribution. The UPGMA groups I and II were divided into subgroups by the Tocher method. The UPGMA group I, for example, were parted into five groups by the Tocher method, in which only genotypes 01 and 24 did not corresponded within both methods. Likewise, the UPGMA Group II were parted into three Tocher groups, whereas only the genotype 09 did not match the corresponding group. Similarities between the Tocher optimization method and the hierarchical unweighted pair group method using arithmetic averages (UPGMA) were also found by Giles et al. [31] and Dubberstein et al. [53]. In relation to the relative contribution of each root trait, as the root diameter has the lowest contribution (6.55%), it should not be considered as a main root trait for *C. canephora* breeding.

4.3. Root Traits, Plant Height and Crop Yield Correlations

The positive correlation between root traits and coffee yield suggests that the more abundant the root systems, higher coffee yield. As the correlations between root traits and plant height were weak or moderate, the root system of *C. canephora* genotypes should not be inferred merely based on plant height. Moreover, the correlation between plant height and coffee yield was weak. According to Carvalho et al. [54], plant height is more related to environmental aspects than to crop yield.

4.4. Main Limitations of the Study

There are a few limitations in this study which should be noted. Firstly, there was no water stress in the assessed coffee trees, as plants were irrigated by drip irrigation. The introduction of any stress may therefore cause differences in crop yield and root distribution. Secondly, neither soil porosity nor soil resistance to penetration have been assessed. It is well known that the soil structure and aggregation are closely related to root distribution and to the potential of water storage in the soil and the extraction by plant roots. Thirdly, there was only one genotype propagated by seeds, which makes it difficult to infer about the differences between genotypes propagated by stem cutting

and by seeds. Future works should therefore fill these gaps in order to consolidate the knowledge about the relationship between root abundance and drought tolerance.

5. Conclusions

In this work, the diversity of root traits in 43 *C. canephora* genotypes and its correlation with plant height and crop yield were analyzed. Most roots were concentrated in the topsoil (0–20 cm) for all assessed genotypes. Considering the 30–60 cm depth, genotypes ID 14, 25, 31, and 32 had more roots than most others coffee genotypes, which suggest they are promising under adverse environmental conditions, such as drought.

Root traits significantly varied within the genotypes propagated by stem cutting, and the seed-propagated genotype (39) had intermediate values, indicating that root development is mostly related to the plant genetic background than to the propagation method.

There were positive correlations between the assessed root traits and both crop yield and plant height. The results of this work may contribute to the overall cultivation of *C. canephora*, especially for crop breeding towards abiotic stress tolerance.

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