







Genetic variability of conilon coffee population from cultivar 'ES8152' based on morphoagronomic variables

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ABSTRACT

This study aimed to analyze the genetic variability of *Coffea canephora* population with 190 genotypes from cultivar 'ES8152', based on morphoagronomic characteristics and vegetation index, to identify the most important characteristics for genetic divergence and compare them with commercial clones. The experiment was installed, in 2019, at the Bananal do Norte Experimental Farm/INCAPER, Cachoeiro de Itapemirim, ES, Brazil. The experiment was carried out in Federer's augmented block design with three blocks, four common treatments (commercial clones A1, LB1, V8 and V12) and 190 regular treatments, genotypes from the seed production field of the conilon coffee cultivar 'ES8152'. At 24 months of age 14 morphoagronomic characteristics and vegetation index were evaluated. Descriptive analysis of the data, the estimation of the Standardized Euclidean Distance (ED) followed by the grouping by the methods of Tocher, UPGMA and principal coordinates, in addition to the relative importance of the characters estimated by the Singh methodology were performed. The most distant genotypes were 62 and 83 (ED=2.620) and the closest were 42 and 160 (ED=0.208). Genotype 83 stood out as the most distant among the others. The optimization and hierarchical groupings allowed the identification of genotypes 15, 81, 107 and 184 as similar to commercial clones. The discard analysis of variables recommended the elimination of the vegetation index and average internode length of the next diversity analysis. Principal coordinate analysis found phenotypic similarity of the genotypes 30, 81, 115, 141 and 163 with the clone V12, of the genotype 119 with the clone A1 and genotype 17 with clone LB1. The study, of morphoagronomic characters, allowed to detection the genetic diversity existing in the materials evaluated, indicating those with phenotypic similarity with the commercial clones, being possible the early identification of promising genotypes, agronomically superior, to start a breeding program for clonal selection, recurrent selection and controlled crosses to maximize heterosis.

Key words: *Coffea canephora*; pre-breeding; genetic diversity; clusters.

1 INTRODUCTION

Coffee is one of the great products of Brazilian agribusiness with expected production of 48,807 thousand bags (60 kg) in an area of 1,824.7 thousand hectares in 2021 (Companhia Nacional de Abastecimento - CONAB, 2021). The Espírito Santo state stands out on the national scenario as the second largest coffee producer, with great emphasis on the conilon coffee. The estimated cultivation area, in Espírito Santo state for 2021, was 400.4 thousand hectares with a production of 14.126 million bags, with 2.986 million bags of arabica coffee and 11.140 million bags of conilon coffee (CONAB, 2021). Major technological advances in Capixaba coffee growing are related to research and rural extension work, carried out by different multidisciplinary and multi-institutional teams. In this context, the clonal (nine) and seed propagated (two) cultivars obtained and made available by the Capixaba Institute of Research, Technical Assistance and Rural Extension (INCAPER) were registered, which constitute the basic genetic material of the planted and renewed crops.

Currently, the main cultivar of *C. canephora* of seminal propagation is 'ES 8152', also known as 'Conquista', developed

by INCAPER and released in 2019. It is a cultivar formed by the recombination of 56 agronomically superior genotypes, in an isolated, for seed production. The main characteristics of this cultivar are broad genetic base, rusticity, adaptation, high productivity, production stability, drought tolerance, rust resistance, high vegetative vigor, good grain processing yield. In addition, it is recommended mainly for small and medium family-based producers (Ferrão RG et al., 2019).

The planting of cultivars propagated by seeds ensures greater natural variability of the species in crops, being considered a simple method and the main strategy for obtaining highly heterozygous offspring (Souza et al., 2021). Conilon coffee (*Coffea canephora*) is an allogamous and diploid species, with gametophytic self-incompatibility (Moraes et al., 2018; Tran et al., 2017). Thus, reproduction by seeds results in a highly diverse population, in which each plant may differ from the others in terms of architecture, shape and size of grains and leaves, maturation pattern and susceptibility to biotic and abiotic stresses (Dubberstein et al., 2020). The genetic diversity existing among and within populations can be measured by the difference among the phenotypic values of their accessions and is obtained in field experiments using

a considerable number of morphological, agronomic and other characteristics (Pontes et al., 2020).

The evaluation of genetic diversity allows the identification of distinct heterotic groups, which, when oriented in controlled crosses, will allow the obtainment of offspring with higher heterosis. Several multivariate methods can be applied for this purpose, such as dissimilarity measures calculated from the Euclidean distance or generalized Mahalanobis distance; clustering methods such as hierarchical (UPGMA) and optimization (Tocher); and dispersion techniques (Cruz; Regazzi; Carneiro, 2012).

Face to importance of cultivars propagated by seeds to the genetic improvement program, this study aimed to evaluate the genetic variability of 190 genotypes from conilon coffee cultivar 'ES8152', propagated by seeds, based on morphoagronomic characteristics and vegetation index, comparing them with commercial superior clones, in addition to identifying the most important characteristics for genetic divergence.

2 MATERIAL AND METHODS

2.1 Genetic materials

The genotypes came from the seed production field of the cultivar 'ES8152', composed of 56 superior clones, from the INCAPER improvement program, planted in row and in an isolated field. The seed field was implemented in 2016/17 at the Bananal do Norte Experimental Farm (FEBN), belonging to the South Center for Research, Development and Innovation (CPDI Sul)/INCAPER in Pacotuba, district of the municipality of Cachoeiro de Itapemirim, Espírito Santo, Brazil. In the first harvest (2019), a seed sample was collected from all plants. These samples were mixed to form a composite sample. Part of the sample (population) was taken to a nursery for the production of seedlings, together with the four clonal witnesses.

Thus, the work consisted of the evaluation of the population with 240 plants from the seed sample composed from cultivar 'ES8152' and four clonal witnesses. The clonal witnesses used were the commercial clones: A1 (108 from 'ES8112-Diamante' cultivar), LB1 (201 from 'ES8122- Jequitibá' cultivar), 23 (V8 from 'ES8142-Vitória' cultivar) and 02/86 (V12 from 'ES8142-Vitória' cultivar).

2.2 Experimental description

The experiment was installed in November 2019 at FEBN, which is located in the south of the Espírito Santo state, Brazil, at latitude 20°45' S, longitude 41°17' W and altitude of 140 meters. The soil is classified as dystrophic Red-Yellow Latosol, Cwa climate with rainy summer and dry winter according to Köpen's classification, mean annual precipitation of 1,200 mm, mean annual temperature of 23 °C and undulating topography.

Experimental design adopted in this study was Federer's augmented block (Federer, 1956) design with three blocks, 240 regular treatments (genotypes) and four common treatments (clonal witnesses), at the spacing of three meters between lines and one meter between plants. The fertilization of planting and conduction followed the fertilization and liming manual for Espírito Santo state (Prezotti et al., 2007) and the cultural and phytosanitary treatments were carried out accordingly with the requirement of the crop, following the current recommendations for conilon coffee (Ferrão RG et al., 2019).

2.3 Agronomic evaluations and vegetation index

At 24 months of age, 190 genotypes of the 240 of the experiment were evaluated, in addition to the four clonal witnesses, with respect to the following characteristics:

CH: Canopy height was measured with a graduated ruler, by the distance between the beginning of the coffee tree canopy and its end (cm);

PH: Plant height was measured with a graduated ruler, by the length of the largest orthotropic branch (cm);

LLB, LMB and LUB: Length of the lower, middle and upper plagiotropic branches, respectively, were measured with a graduated ruler, by the largest plagiotropic branches in the lower, middle and upper portions of the coffee tree (cm);

IL: Average internode length of the largest orthotropic branch, was estimated by the ratio CH/NN (cm);

SBD: Stem base diameter was measured with a precision digital caliper (0.01 mm) in the intermediate position of the soil up to the first plant node perpendicular to the planting line (mm);

CD: Canopy diameter of the coffee tree was measured with a graduated ruler, by the length of the greatest distance of the end of the branches that compose the coffee tree canopy, perpendicular to the planting line (cm);

NDVI: Normalized Difference Vegetation Index was measured with a PlantPen NDVI-300 portable sensor (Photon Systems Instruments PSI, Drásov, Czech Republic), using two leaves of the third or fourth pair, from the plagiotropic branch, from the middle position of the plant (und);

NLLB, NLMB and NLUB: Number of leaves from lower, middle and upper plagiotropic branches, respectively, were obtained by counting the number of leaves in the branches in which LLB, LMB and LUB were evaluated (und);

NF: Number of fruits in the plant was obtained by counting the fruits present in the plant from plagiotropic branches used to evaluate LLB, LMB and LUB (und).

NN: Number of nodes was obtained by directly counting nodes in the largest orthotropic branch (und);

NPB: Number of plagiotropic branches, was obtained by directly counting the number of plagiotropic branches of the coffee tree (und).

2.4 Statistical analysis of data

Descriptive analysis of the original data was performed to estimate the phenotypic pattern of cultivar 'ES8152' in comparison with commercial clones.

Based on the set of characteristics (15) and genotypes (194), the matrix of genetic distances was estimated using the Standardized Euclidean Distance (ED), and the grouping of genotypes by the Tocher optimization methods (Rao, 1952), hierarchical UPGMA and principal coordinate analysis. The cophenetic correlation coefficient between the graphical distance matrix and the original distance matrix was estimated. And the relative importance of characters in genetic divergence was estimated by the methodology proposed by Singh (1981). In the principal coordinates analysis, the number of axes necessary to preserve at least 60% of the variance of the original dataset was used.

Statistical analyzes were performed in the computer applications R (R Core Team, 2019) and GENES (Cruz, 2016).

3 RESULTS

Descriptive analysis of the data (Table 1) demonstrates that the mean and median of the evaluated characteristics are close and, therefore, the data are close to the normal distribution, except for the NF characteristic, in which the median was zero. Several genotypes did not show production

at 24 months of age due to the longer period of juvenility of seminal seedlings compared to clonal seedlings. A higher standard deviation and data variation index were observed for the NF characteristic, demonstrating a greater variability in the observations.

The means of commercial clones showed values between the maximum and the minimum observed in the data set under study and clones A1, LB1 and V8 presented the highest values for NF. The observed mean of clone V12 was lower than the mean of the data set for the characteristics LLB, LMB, LUB, IL, CD and NLUB. Clone V8 mean was lower than the data set mean for IL and NLUB characteristics. The mean of clone LB1 was also lower for the IL characteristic and equal to the mean of the NDVI standard of the dataset.

Based on the genetic distance matrix, the largest and smallest statistical distances were, respectively, between the pairs of genotypes 62 and 83 (2.620) and 42 and 160 (0.208) (Table 2). In the grouping of genotypes by the Tocher Optimization method (Table 3) and UPGMA (Figure 1), genotype 83 also stood out as the most divergent.

By Tocher's grouping, the most individuals in the studied population were allocated in the same group (91.05 % in group 1). The clonal witnesses, clones A1 and V12 were also in group 1, and clones LB1 and V8 in group four together with genotype 107. Individuals 83, 11, 67 and 107 were the most dissimilar in the population, including in relation to clonal witnesses.

Table 1: Descriptive analysis of the population of 190 genotypes of cultivar 'ES8152' and four clonal witnesses, evaluated for 15 characteristics at 24 months of age at the Bananal do Norte Experimental Farm, INCAPER.

Characteristics ¹	Maximum	Minimum	Median	Mean	SD ²	VI (%) ³	A1	LB1	V8	V12
CH (cm)	140.000	41.000	100.000	99.68	17.936	1.305	126.000	126.800	120.580	121.500
PH (cm)	182.000	87.000	139.000	137.500	17.759	0.937	146.830	143.200	146.830	136.750
LLB (cm)	106.000	40.000	70.000	70.500	12.207	1.256	84.670	82.600	77.920	68.080
LMB (cm)	97.500	33.000	50.000	52.090	12.313	1.715	61.170	63.700	52.420	51.670
LUB (cm)	39.000	8.500	16.00	16.230	4.218	1.886	17.420	17.800	34.500	11.080
IL (cm)	7.5000	3.090	5.330	5.350	0.811	1.099	6.760	5.160	5.180	5.220
SBD (mm)	69.000	32.000	49.000	49.580	7.928	1.160	52.330	58.800	61.000	51.170
CD (cm)	235.000	93.000	152.000	153.400	26.476	1.252	185.080	174.20	172.580	151.580
NDVI (und)	0.689	0.497	0.623	0.620	0.030	0.347	0.624	0.620	0.627	0.623
NLUB (und)	20.000	3.000	6.000	6.820	1.965	2.089	6.830	7.000	6.000	6.000
NLMB (und)	114.000	6.000	18.000	19.200	9.236	3.490	20.000	23.000	20.000	27.000
NLLB (und)	123.000	5.000	27.000	32.680	21.021	4.666	38.330	41.000	47.000	44.000
NF (und)	206.000	0.000	0.000	9.070	23.989	19.183	143.000	206.000	115.000	39.000
NN (und)	30.000	9.000	19.000	18.790	3.167	1.222	19.000	25.000	23.000	23.000
NPB (und)	152.000	40.000	104.000	103.500	18.782	1.316	121.500	136.00	131.000	132.000

¹Canopy height (CH); Plant height (PH); Length of the lower, middle and upper plagiotropic branches (LLB, LMB and LUB); Average internode length (IL); Stem base diameter (SBD); Canopy diameter (CD); Normalized Difference Vegetation Index (NDVI); Number of leaves from lower, middle and upper plagiotropic branches (NLLB, NLMB and NLUB); Number of fruits (NF); Number of nodes (NN); Number of plagiotropic branches (NPB). ²Standard deviation (SD); ³Variation index (VI).

Table 2: Description of the ten largest and smallest genetic distances among the genotypes of the cultivar 'ES8152' and clones A1, LB1, V8 and V12, obtained by the Standardized Euclidean Distance matrix, from morphoagronomic characteristics and vegetation index. Bananal do Norte Experimental Farm, INCAPER.

Greater genetic distances	Accesses	Smallest genetic distances	Accesses
2.620	62 e 83	0.208	42 e 160
2.568	61 e 83	0.213	111 e 155
2.518	83 e 128	0.217	145 e 161
2.510	53 e 83	0.218	35 e 160
2.464	63 e 83	0.229	6 e 35
2.399	64 e 83	0.251	137 e 179
2.337	62 e 79	0.257	88 e 145
2.307	83 e 93	0.260	35 e 42
2.252	83 e 129	0.261	34 e 115
2.247	61 e 79	0.264	146 e 164

Table 3: Genotypes grouping of the cultivar 'ES8152' and clones A1, LB1, V8 and V12 by Tocher's optimization method, based on the Standardized Euclidean Distance matrix, from morphoagronomic characteristics and vegetation index. Bananal do Norte Experimental Farm, INCAPER.

Group	N	Accesses
1	175	1. 2. 3. 4. 6. 8. 9. 10. 12. 13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25. 27. 28. 29. 30. 31. 32. 33. 34. 35. 36. 37. 38. 40. 41. 42. 43. 44. 45. 46. 47. 48. 49. 50. 51. 52. 54. 55. 56. 57. 58. 59. 60. 65. 66. 68. 69. 70. 71. 72. 73. 74. 75. 76. 77. 78. 81. 82. 84. 85. 86. 87. 88. 89. 90. 91. 92. 93. 94. 95. 96. 97. 98. 99. 100. 101. 102. 103. 104. 105. 106. 108. 109. 110. 111. 112. 113. 114. 115. 116. 117. 118. 119. 120. 121. 122. 123. 124. 125. 126. 127. 130. 131. 132. 133. 134. 135. 136. 137. 138. 139. 140. 141. 142. 143. 144. 145. 146. 147. 148. 149. 150. 151. 152. 153. 154. 155. 156. 157. 158. 159. 160. 161. 162. 163. 164. 165. 166. 167. 168. 169. 170. 171. 172. 173. 174. 175. 176. 177. 178. 179. 180. 181. 182. 183. 184. 185. 186. 187. 188. 189. 190. A1. V12
2	7	53. 61. 62. 63. 64. 128. 129
3	6	5. 7. 26. 39. 79. 80
4	3	LB1. V8. 107
5	2	11. 67
6	1	83

In the UPGMA cluster was verified a cophenetic correlation coefficient of 0.719 and the separation of clone V12 of the other commercial clones. Clone V12 was grouped initially with genotype 81. Clones LB1 and V8 were close, being later grouped in the same group with clone A1 followed by genotypes 15 and 184. The clusters formed by Tocher's optimization and the UPGMA hierarchical method allowed the identification of genotypes 15, 81, 107 and 184 as the closest to commercial clones.

The relative importance analysis of the characteristics for genetic divergence (Table 4) shows that CD, NF, NLLB, NPB, CH and PH accumulated the largest proportion of variability with 21.979, 18.044, 13.856, 11.061, 10.086 and 9.889 of the variation, respectively. These characteristics added up to about 75% of variation in the data set, thus being the most important in the selection process. The NDVI and IL characteristics presented zero and 0.021 of data variability,

being recommended to discard these analyzes in future diversity studies.

The principal coordinate analysis (Table 5) shows the distribution of genotypes in a two-dimensional plane. Due to the accumulation of 64.7% variation in the first three axes, the demonstration of the dispersion of individuals combining the three axes was adopted (Figure 2). It was found that in the three possible combinations, the most distant accesses were distributed at the ends of the graph and the closest ones in the interior. In the axes combination one and two, genotypes 20, 25, 50 and 119 were located close to clone A1, genotypes 17 and 80 were found around LB1, genotypes 30, 34, 81, 115, 124 and 141 close to V12 and genotypes 28, 76 and 135 close to V8. In the axes combination one and three, genotypes 70, 119 and 135 were found close to clone A1. Clones LB1 and V8 were close and neighbors to genotypes 17, 50 and 178, clone V12 was adjacent to genotypes 30, 32, 49, 117, 144,

163, 169 and 171. In the axes combination two and three, clone A1 was close to genotypes 46, 59, 101, 119 and 183, clone LB1 next to genotypes 17, 27, 88, 89, 120, 152 and 182, clone V12 next to genotypes 30, 92, 104, 112, 115, 141 and 163 and clone V8 next to genotypes 19, 74, 86, 110, 114, 145 and 150.

4 DISCUSSION

The Variation Index values (Table 1) were close to those estimated by Senra et al. (2020) in the evaluation of 38 traits from 323 accessions of the active germplasm bank of the INCAPER at FEBN, demonstrating similar experimental control, with good precision in the evaluation of morphoagronomic characters, at 24 months of age, of genotypes of cultivar 'ES8152' and clonal witnesses. The SBD means obtained in this study were close to those of the active germplasm bank of the INCAPER (Senra et al., 2020), and higher for the characteristics CD, LLB, LMB, LUB, NLMB and NLLB.

The greatest statistical distance observed was greater than those estimated in previous studies based on phenotypic

and, or, molecular data from the Incaper research program (Ferrão et al., 2009; Ferrão et al., 2017; Ferrão LFV et al., 2019; Ferrão et al., 2021; Fonseca et al., 2006; Giles et al., 2018; Senra et al., 2020; Souza et al., 2021), demonstrating the presence of genetic variability to be explored. The genetic materials identified as divergent, in the UPGMA, Tocher and principal coordinates clusters, must be monitored for their agronomic characteristics over at least four harvests for selection and use per se and, or, in hybridizations in the INCAPER breeding program. The obtaining these results, in the initial phase of experimentation, are important to guide selection and recombination strategies focused on the formation of heterotic groups for controlled crosses. Additionally, the direct selection of superior genotypes is a fundamental strategy in the composition of trials to determine the value of cultivation and use (VCU), followed by the launch of new cultivars. According to Borém and Miranda (2013) and Ferrão MAG et al. (2019), crosses involving genetically different parents can produce a high heterotic effect and the breeder can obtain hybrids or clones superior to the paternal.



Figure 1: Genotypes grouping of the cultivar 'ES8152' and clonal witnesses A1 (191), LB1 (192), V8 (194) and V12 (193) by UPGMA hierarchical method, based on the Standardized Euclidean Distance matrix, from morphoagronomic characteristics and vegetation index. Bananal do Norte Experimental Farm, INCAPER.

Table 4: Relative contribution of 15 characteristics evaluated in 190 conilon coffee genotypes of cultivar 'ES8152' and clones A1, LB1, V8 and V12, based on Singh (1981)'s method, using Standardized Euclidean Distance (D^2). Bananal do Norte Experimental Farm, INCAPER.

Characteristics ¹	Relative contribution (%)
CH (cm)	10.09
PH (cm)	9.89
LLB (cm)	4.67
LMB (cm)	4.75
LUB (cm)	0.56
IL (cm)	0.02
SBD (mm)	1.98
CD (cm)	21.98
NDVI (und)	0,00
NLUB (und)	0.12
NLMB (und)	2.67
NLLB (und)	13.86
NF (und)	18.04
NN (und)	0.31
NPB (und)	11.06

¹Canopy height (CH); Plant height (PH); Length of the lower, middle and upper plagiotropic branches (LLB, LMB and LUB); Average internode length (IL); Stem base diameter (SBD); Canopy diameter (CD); Normalized Difference Vegetation Index (NDVI); Number of leaves from lower, middle and upper plagiotropic branches (NLLB, NLMB and NLUB); Number of fruits (NF); Number of nodes (NN); Number of plagiotropic branches (NPB).

The cophenetic correlation coefficient was high (0.719) and higher than that estimated by Ferrão et al. (2021) with a study of 562 genotypes of the active germplasm bank, demonstrating low distortion between the matrix of graphic distances and real distances.

Based on the results of the relative importance of the characters for genetic diversity, it is recommended to discard the NDVI and IL characters, based on the evaluation of the first harvest. However, recent studies have used the vegetation index in research on coffee cultivation as the relationship between meteorological variables and vegetation index (Mota et al., 2020), the different phenological phases of coffee (Nogueira; Moreira; Volpato, 2018), monitoring of coffee maturation to determine the best moment of the harvest (Nogueira et al., 2021). In addition, Silva et al. (2021) suggest the use of NDVI, as an auxiliary character, in the selection of coffee genotypes from six months of age.

The principal coordinates analysis corroborated with the confirmation of the proximity of genotype 81 with clone V12, because the analysis of axes one and two combined with the UPGMA grouping. In the analysis of the three axes, the stability of proximity of genotypes 30, 115, 141 and 163 and clone V12, genotype 119 with clone A1 and genotype 17 with LB1 was observed.

Studies of this nature are important for the genetic improvement of coffee, as they allow the early identification of promising genotypes, agronomically superior, to initiate a program of clonal selection, recurrent selection and guide controlled crosses to maximize heterosis.

Table 5: Relative contribution of axes in principal coordinate analysis based on 15 characteristics evaluated in 190 conilon coffee genotypes of cultivar 'ES8152' and clones A1, LB1, V8 and V12. Bananal do Norte Experimental Farm, INCAPER.

Axes	Eigenvalues axes	Proportion explained by axes	Adjusted accumulated value
1	33.037	0.415	0.415
2	11.252	0.141	0.556
3	7.213	0.091	0.647
4	5.385	0.068	0.714
5	4.659	0.058	0.773
6	4.422	0.056	0.828
7	3.793	0.048	0.876
8	2.767	0.035	0.911
9	2.225	0.028	0.938
10	1.486	0.019	0.957
11	1.054	0.013	0.970
12	0.977	0.012	0.983
13	0.695	0.009	0.991
14	0.648	0.008	0.999
15	0.042	0.001	1.000

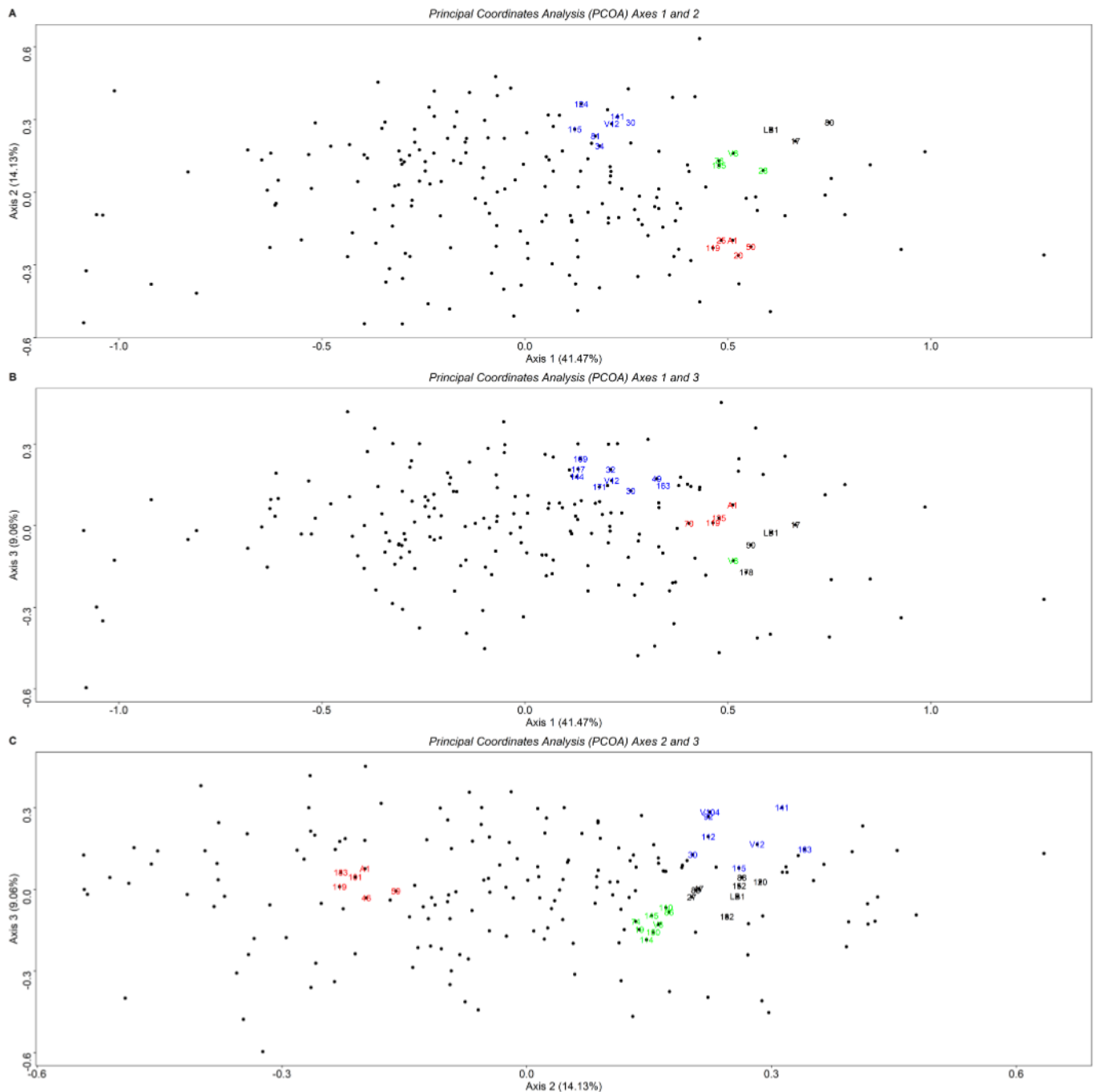


Figure 2: Graphic dispersion of genotypes of the population from cultivar ‘ES8152’ and clonal witnesses A1 (red), LB1 (black), V8 (green) and V12 (blue), obtained by principal coordinate analysis method, based on morphoagronomic characteristics and vegetation index. A) Principal coordinate analysis Axes 1 and 2. B) Principal coordinate analysis Axes 1 and 3. C) Principal coordinate analysis Axes 2 and 3. Bananal do Norte Experimental Farm, INCAPER.

5 CONCLUSIONS

The study, of morphoagronomic characters, allowed to detection the genetic diversity existing in the population of 190 genotypes from conilon coffee cultivar ‘ES8152’, propagated by seeds, indicating those with phenotypic similarity with the commercial clones.

The genotypes 62 and 83 stood out with the largest genetic distance, genotypes 42 and 160 as the most similar and the genotype 83 as the most divergent in the study.

The characters canopy diameter, number of fruits and number of leaves from lower plagiotropic branches were the ones that most contributed to genetic diversity.

The genotypes 15, 17, 30, 81, 107, 115, 119, 141, 163 and 184 showed to be similar to the clonal witnesses.

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7 AUTHORS' CONTRIBUTION

JFBS and JAS wrote the manuscript and performed the experiment, MAGF contributed the discussion of the results and writing of the manuscript, MDDE supervised the experiment, ISM and KMF experimental evaluations.

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