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Genetic Variability of Access of the Active Germplasm Bank of Coffea canephora of Incaper in Southern Espírito Santo

Joao Felipe de Brites Senra^{1*}, Maria Amélia Gava Ferrao², Rodolfo Ferreira de Mendonça³, Aymbiré Francisco Almeida da Fonseca², Romário Gava Ferrao⁴, Paulo Sérgio Volpi¹, Abraão Carlos Verdin Filho¹, Marcone Comério¹ and Matheus Wandermurem da Silva⁵

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*Corresponding authors:

⊠ JFB. Senra joao.senra@incaper.es.gov.br

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ABSTRACT

This study aimed to analyze the genetic variability of 323 accessions of the Active Germplasm Bank (BAG) of Coffea canephora of the Institute for Research, Technical Assistance and Rural Extension of Espírito Santo (Incaper) using 38 quantitative phenotypic characters. The standardized average Euclidean distance between the accessions was estimated to generate a statistical distance matrix and, from this, the groupings were performed using the Tocher and UPGMA. Concerning the studied accessions, the amplitude of the data set for each characteristic, and the possibility of selection were visualized. The accuracy of data collection was verified by the Variation Index with values below 10% for most of the characters, except for characters such as number of rosettes in the upper plagiotropic branch, number of grains in the smallest orthotropic branch, and number of grains per rosette on the upper plagiotropic branch. Using the Tocher method, 25 groups were recognized, 10 of which were formed by only one accession. The hierarchical grouping highlighted the lack of duplicates and accessions 173 (ES 1-B) as the most genetically distant. The analysis of the relative contribution of each character distinguished fresh matter and dry matter of orthotropic branches thrown by plants susceptible to pruning as fundamental for the differentiation of accessions and important in future studies of diversity as they are responsible for about 83% of the phenotypic variability of the study. There were no duplicates among the evaluated accessions and there are heterotic groups and distinct accessions in the BAG that can be used in hybridization programs or per se to obtain new cultivars. The pairs of the most similar and dissimilar accessions were 45 (148/86) and 320 (IAC37) with a statistical distance of 0.0713 and 173 (ES 1-B) and 270 (403-Marilândia) with a distance of 0.4765. respectively.

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Introduction

Coffee production is one of the pinnacles of the agricultural economy in the world which occupy about 11 million hectares and more than 80

countries involved in its production (Denoeud et al., 2014), structuring a billion-dollar productive chain (ICO, 2018). The international coffee trade is concentrated in Coffea arabica (Arabica

¹ Development and Innovation Institute, Technical Assistance and Rural Extension of Espírito Santo, Rodovia Joao Domingo Zago, Brazil

Brazilian Agricultural Research Corporation Institute, Technical Assistance and Rural Extension of Espírito Santo,

Itapemirim City Hall, ES. Former Pos Doctor Junior CNPq fellow and Consorcio Pesquisa Café in INCAPER, Brazil

⁴ Technical Assistance and Rural Extension of Espírito Santo and research coordinator of Brazilian College Multivix,

Research Coffee Consortium Scholarship of Southern Center, Development and Innovation Institute, Technical Assistance, and Rural Extension of Espírito Santo, Brazil

coffee), 60% of the market, and Coffea canephora (Conilon/Robusta coffee), 40% of the market (ICO, 2018). Brazil is the largest producer and exporter of coffee (Coffea spp.) accounting for 37% of world production (USDA, 2019). The consumption of Conilon coffee, mainly in the form of soluble coffee, increases worldwide, and the main consumers are the United Kingdom, the Philippines, China, Russia, and the United States (USDA, 2019). The production of Brazilian coffees (Conilon and Arabica) was estimated at 49.97 million bags of 60 kg in 2019 and is expected to reach 59.58 million bags in 2020 (Companhia Nacional de Abastecimento, 2020). For Conilon coffee approximately 16 million bags are estimated for 2020 (Companhia Nacional de Abastecimento, 2020).

The genus Coffea has 124 species (Davis et al., 2011) and these occur naturally in tropical Africa, the islands of the Indian Ocean (Madagascar, Comoros, and the Mascarene Islands), Asia, and Australia (Davis et al., 2011). Wild forms of C. canephora occur in much of tropical humid Africa (Davis et al., 2006) ranging from Guinea to Uganda (Solórzano et al., 2017). Berthaud (1986) was the first author to describe a genetic diversity of the genus Coffea, identifying two distinct genetic groups based on their centers of diversity: The Guinean group, formed by West African genotypes (Guinea, Liberia, and Côte d'Ivoire); and the Congolese group, formed by genotypes from Central Africa (Ferrao et al., 2015). The first molecular study of the genetic diversity of C. canephora was reported in 1980 (Montagnon et al., 1992; Musoli et al., 2009; Cubry et al., 2013) and this one also identified the Congolese and Guinean groups (Solórzano et al., 2017).

The Congolese group was split into five subgroups SG1, SG2, B, C, and UW (Musoli *et al.*, 2009; Ferrao *et al.*, 2019), and only a small part of this great diversity (SG1 and SG2) is used in the improvement of the current program. The SG1 subgroup is formed by genotypes from the region of Benin to Gabon. These are known as Conilon coffee and are more adapted to Brazil and present in the main national varieties (Alkimim *et al.*, 2018). Subgroups SG2 (from the Democratic Republic of Congo), B (from the Central African Republic), and C (from

Cameroon) are the genotypes known as Robusta coffee (Alkimim *et al.*, 2018), these coffee trees are tall, vigorous, with large leaves and fruits, resistant to coffee rust and more susceptible to drought (Marraccini *et al.*, 2012).

It is estimated that there are about 30,288 accessions of coffee (Coffea spp.) preserved in collections in germplasm banks ex-situ worldwide (Laliberté et al., 2012; Bramel et al., 2017). These accessions are preserved in institutions that face national financial difficulties to maintain and reproduce genetic diversity (Lebot et al., 2020). In Brazil, the main germplasm collections of C. canephora are found in governmental institutions such as the Agronomic Institute of Campinas (IAC), Embrapa Rondônia, and the Institute for Research. Technical Assistance and Rural Extension of Espírito Santo (Incaper) (Souza et al., 2013). The accessions maintained by the IAC are composed mainly of materials from Africa, after the FAO introduced expeditions (Silvestrini et al. 2008). However, Incaper and Embrapa Rondônia have a significant number of accessions obtained in production fields.

The analysis of genetic variability between accessions of a species is vital for the identification of promising genotypes and/or distinct ones, in addition to enabling the grouping of these genotypes to obtain homogeneity within group each and heterogeneity between the groups (Carmona et al., 2015). Most studies conducted in ex-situ collections use standardized morpho-agronomic descriptors and molecular markers to assess genetic diversity (Cosme et al. 2016; Anagbogu et al. 2019). Multivariate techniques aggregate multiple information simultaneously and its use is common in Conilon coffee (Rocha et al., 2014; Dalcomo et al., 2015; Silva et al., 2015; Covre et al., 2016). Genetic diversity is one of the key elements for any breeding program to be effective (Rahman and Islam, 2020). Activities such as hybridization will only be efficient through the selection of superior and divergent parents (Archana et al., 2018). Additionally, diversity studies assist in the maintenance and the efficient use of germplasm banks (Rabbani et al., 1988), a fundamental activity for the maintenance of the Coffea genus, especially in this era of climate change. According to Davis *et al.* (2019), about 60% of the species of the genus *Coffea* are threatened with extinction and 45% are not in any germplasm collection.

Incaper stands out in the development of several varieties of Conilon/Robusta coffee and has been conducting a solid genetic improvement program since 1985. Over this period, a significant number of genotypes with superior characteristics have been selected and preserved in the Active Germplasm Bank (BAG) of the institution. These genotypes represent the raw material of the Incaper's genetic improvement program and enabled the development of 11 Conilon coffee cultivars that meet the various technical demands of the State of Espírito Santo coffee production. The objective of this work was to analyze the genetic variability of 323 accessions of Incaper's BAG using 38 quantitative phenotypic characters related to plant architecture, production, and maturation, applying the standardized average Euclidean distance and the hierarchical grouping UPGMA (Unweighted Pair Group Method using Arithmetic Averages) and Tocher optimization methods.

Materials and Methods

The Active Germplasm Bank (BAG) of C. canephora of Incaper is established in three Experimental Units of Incaper, indistinct and representative regions of the local culture in the State, aiming, in addition to maintenance, the characterization of the accessions for biotic and abiotic factors. The data collection of this work took place in the BAG established in the Experimental Farm of Bananal do Norte (FEBN), belonging to the Southern Center for Research, Development, and Innovation (CPDI Sul) of Incaper in Pacotuba, district of the municipality of Cachoeiro de Itapemirim. The FEBN is located at latitude 20°45 'S and longitude 41°17' W, in the south of the State of Espírito Santo, Brazil, at 140 meters of altitude. The soil is classified as dystrophic Red-Yellow Latosol, climate Cwa with rainy summer, and dry winter according to the Köpen classification. The region presents annual rainfall of 1,200 mm, an average annual temperature of 23 °C and undulating topography. The BAG was planted in this location in May 2017, at a spacing of 3

meters between lines and 1.5 meters between with 500 accessions and plants three plants/accession, surrounded by a borderline different genotypes. Fertilization management follows the recommendation of the fertilization and liming manual for the State of Espírito Santo (Prezotti et al., 2013). Cultural and phytosanitary treatments were carried out according to the requirement of the crop following the current recommendations for Conilon coffee (Ferrao et al., 2017b).

During the first harvest in 2019, 323 accessions were evaluated using 38 quantitative characters that describe the architecture of the plant, productive potential, and fruit maturation. Characteristics evaluated: Number of orthotropic branches (NR) (unit); Orthotropic branches thrown by plants susceptible to pruning (ROL) (unit); Length of the smallest orthotropic branch (MERO) (cm); Length of the largest orthotropic branch (MARO) (cm); Stem base diameter (DBC) (mm); Number of nodes in MERO (NMERO) (unit); Number of nodes in MARO (NMARO) (unit); Number of plagiotropic branches in the plant (NRP) (unit); Length of the lower (CRPI) (cm), medium (CRPM) (cm) and upper (CRPS) plagiotropic branch (CRPI) (cm) that represents the structure of the plant; Number of nodes in the lower (NRPI) (unit), medium (NRPM) (unit) and upper (NRPS) (unit) plagiotropic branches; Number of leaves released in the lower (NFPI) (unit), medium (NFPM) (unit) and upper (NFPS) (unit) plagiotropic branches; Larger diameter of the coffee tree crown in projection towards the planting line (DC) (cm); Length of internodes of smallest orthotropic branch, (CEMERO) (cm); Length of internodes of the largest orthotropic branch, MARO (CEMARO) (cm); Length of internodes in the lower plagiotropic branch (CERPI) (cm), medium (CERPM) (cm) and upper (CERPS) (cm); Fresh matter ROL (MFROL) (g); Dry matter ROL (MSROL) dehydrated in an oven with forced air circulation at 65 °C until reaching constant weight (g); number of rosettes in the lower plagiotropic branch (NROPI) (unit), medium (NROPM) (unit), upper (NROPS) (unit): number of grains in the largest (NGMARO) and smallest (NGMERO) (unit) (unit) orthotropic branch; number of grains per rosette

in the lower plagiotropic branch (NGRPI) (unit), medium (NGRPM) (unit), upper (NGRPS) (unit); percentage of green (V), ripe (M) and dry (S) grains based on a random sampling of 100 grains from the plant; Weight of coffee harvested per plant (Weight) (kg); percentage of grain floats based on a sample of 100 ripe grains per plant.

Based on the evaluated characteristics, the average Euclidean standardized distance (DEMP) between the accessions was estimated generating a statistical distance matrix. Based on the distance matrix, clusters were performed using the Tocher optimization method (Rao, 1952) hierarchical grouping UPGMA. The relative importance of the characters concerning the genetic divergence was estimated by the methodology proposed by Singh (1981). All statistical analyzes described were performed using the computer application GENES (Cruz, 2013; Cruz, 2016) and R (Team 2019).

Results and Discussion

To characterize the accessions of the BAG, summarized in Table 1, the amplitude of the data set for each character, and the selection possibility was visualized. The accuracy of data collection is verified by the Variation Index with values below 10% for most characters, except for NROPS, NGMERO, and NGRPS. Greater production was observed in the first harvest of accessions 111 and 222, which are component clones of the cultivars Marilândia ES8143 (clone 405) and Diamante ES8112 (clone 108), respectively. Accession 111 was also distinguished by uniform maturation, which is a fundamental factor for obtaining a better quality product. The results of the multivariate analysis. based on the standardized average Euclidean distance (matrix of statistical distances not shown), showed important variability. The closest and farthest accessions pairs were 45 (148/86) and 320 (IAC37) with a statistical distance of 0.0713 and 173 (ES 1-B) and 270 (403-Marilândia) with a distance of 0.4765, respectively. It is worth noting that accession 173 also presented the greatest distances concerning all other studied accessions. Figure 1 illustrates the grouping of 323 accessions according to the UPGMA method, highlighting the lack of duplicates and accessions 173 as the most distant genetically.

Using the Tocher method, a cluster including 25 groups was obtained (Table 2), the last 10 being formed by only one genotype, such as 22 (ES 31/86), 173 (ES 1-B), 321 (ES IAC38), 77 (154/89), 169 (ES 4-B), 217 (ES 186 / 87-1), 61 (ES 87/87), 221 (ES PP103), 115 (ES44 / 89) and 78 (ES 161/89), belonging to groups 16 to 25, respectively. To support the selection of the best accessions and groups, the average of the 25 groups was estimated (Table 3). The genetic distance between the parents is indicative of progenies with greater heterotic effect (Falconer, 1981), however, parallel to genetic divergence, the choice of parents must consider their performance per se (Souza et al., 2005). 173 Accessions showed a good initial performance in the field, standing out as a promising alternative to be used in the future as a paternal in controlled crosses or directly in the composition of new clonal cultivars. The analysis of the relative contribution of each character (Table 3), distinguished MFROL and MSROL as fundamental for the differentiation of accessions and important in future studies of diversity as they are responsible for about 83% of the phenotypic variability of the study. According to Cruz et al. (2012), the characters that are dispensable in studies of genetic divergence include those that are relatively nonvariant among the individuals studied and that are redundant because they are correlated with others. Genetic divergence studies with elite materials from Incaper's breeding program have shown important variability. In the first study, Fonseca (1999) and Fonseca et al. (2006) analyzed by different multivariate procedures the structure of the first three Conilon coffee cultivars indicated for the State of Espírito Santo, composed of 32 clones. The relation between the highest and lowest observed value of generalized Mahalanobis distance was of the order of 130.18, demonstrating a genetic between accessions variability and possibility of selecting the most divergent.

Table 1. Average characterization of 323 accessions of *C. canephora* based on the descriptive analysis of 38 characters evaluated at the Active Germplasm Bank (BAG), Experimental Farm of Bananal do Norte, Incaper, Cachoeiro do Itapemirim, Espírito Santo, Brazil.

Characters 1	Average	Minimum	Maximum	IV^2	Variance	Standard Deviation
NR (unit)	10.66	2.00	40.00	3.11	35.49	5.96
ROL (unit)	6.84	0.00	36.00	4.77	34.33	5.86
MERO (cm)	88.37	10.00	150.00	1.79	808.21	28.43
MARO (cm)	113.94	22.00	225.00	0.96	388.02	19.70
DBC (mm)	50.18	6.10	101.58	1.60	207.00	14.39
NMERO (unit)	16.13	1.00	59.00	2.77	64.42	8.03
NMARO (unit)	23.75	11.00	65.00	1.91	66.31	8.14
NRP (unit)	116.15	47.00	209.00	1.52	1005.17	31.70
CRPI (cm)	55.65	7.00	99.00	1.46	212.37	14.57
CRPM (cm)	54.71	18.00	93.00	1.21	141.84	11.91
CRPS(cm)	31.77	8.00	67.50	2.04	135.71	11.65
NRPI (unit)	14.75	2.00	56.00	2.35	38.88	6.24
NRPM (unit)	14.73	4.00	38.00	1.50	15.81	3.98
NRPS(unit)	8.24	1.00	32.00	2.38	12.47	3.53
NFPI (unit)	17.64	0.00	113.00	4.04	163.90	12.80
NFPM (unit)	18.68	0.00	87.00	3.15	112.02	10.58
NFPS (unit)	13.87	2.00	42.00	2.41	36.02	6.00
DC (cm)	137.62	45.00	200.00	0.96	562.75	23.72
CEMERO (cm)	6.28	0.44	18.33	2.10	5.63	2.37
CEMARO (cm)	5.22	0.76	11.84	1.78	2.78	1.67
CERPI (cm)	4.16	0.20	26.00	2.31	2.99	1.73
CERPM (cm)	3.87	1.18	6.89	1.28	0.80	0.89
CERPS (cm)	4.08	1.56	15.00	1.81	1.77	1.33
MFROL (g)	210.33	0.00	1665.00	6.14	53125.16	230.49
MSROL (g)	76.12	0.00	624.00	6.71	8318.96	91.21
NROPI (unit)	4.37	0.00	18.00	4.98	15.29	3.91
NROPM (unit)	2.51	0.00	13.00	6.12	7.60	2.76
NROPS (unit)	0.24	0.00	10.00	27.98	1.44	1.20
NGMARO (unit)	33.10	0.00	266.00	7.55	2010.89	44.84
NGMERO (unit)	13.14	0.00	167.00	11.28	707.85	26.61
NGRPI (unit)	44.67	0.00	571.00	7.38	3514.05	59.28
NGRPM (unit)	24.05	0.00	317.00	8.21	1258.61	35.48
NGRPS (unit)	0.40	0.00	44.00	45.67	10.74	3.28
V (%)	20.22	0.00	73.00	4.11	223.14	14.94
M (%)	38.19	0.00	93.00	3.40	543.09	23.30
S (%)	24.87	0.00	100.00	4.57	416.34	20.40
Weight (Kg)	1.38	0.00	8.48	5.51	1.86	1.36
Float (%)	8.34	0.00	60.00	7.36	121.50	11.02

¹Number of orthotropic branches (NR) (unit), orthotropic branches thrown by plants susceptible to pruning (ROL) (unit), length of the smallest orthotropic branch (MERO) (cm), length of the largest orthotropic branch (MARO) (cm), stem base diameter (DBC) (mm), number of nodes in MERO (NMERO) (unit), number of nodes in MARO (NMARO) (unit), number of plagiotropic branches in the plant (NRP) (unit), length of the lower plagiotropic branch (CRPI) medium (CRPM) and upper (CRPS) (cm), number of nodes in the lower (NRPI) medium (NRPM) and upper (NRPS) plagiotropic branches, number of leaves released in the lower (NFPI) (unit), medium (NFPM) (unit) and upper (NFPS) (unit) plagiotropic branches, larger diameter of the coffee tree crown in projection towards the planting line (DC) (cm), length of internodes of the smallest orthotropic branch, MERO (CEMERO) (cm), length of internodes of the largest orthotropic branch, MARO (CEMARO) (cm), length of internodes in the lower plagiotropic branch (CERPI) (cm), medium (CERPM) (cm) and upper (CERPS) (cm), fresh matter ROL (MFROL) (g), dry matter ROL (MSROL), number of rosettes in the lower plagiotropic branch (NROPI) (unit), medium (NROPM) (unit), upper (NROPS) (unit), number of grains in the largest (NGMARO) (unit) and smallest (NGMERO) (unit) orthotropic branch, number of grains per rosette in the lower plagiotropic branch (NGRPI) (unit), medium (NGRPM) (unit), upper (NGRPS) (unit), percentage of green (V), ripe (M) and dry (S) grains based on a random sampling of 100 grains from the plant; Weight of coffee harvested per plant (Weight) (Kg); percentage of grain floats based on a sample of 100 ripe grains per plant (Float).

² Variation Index

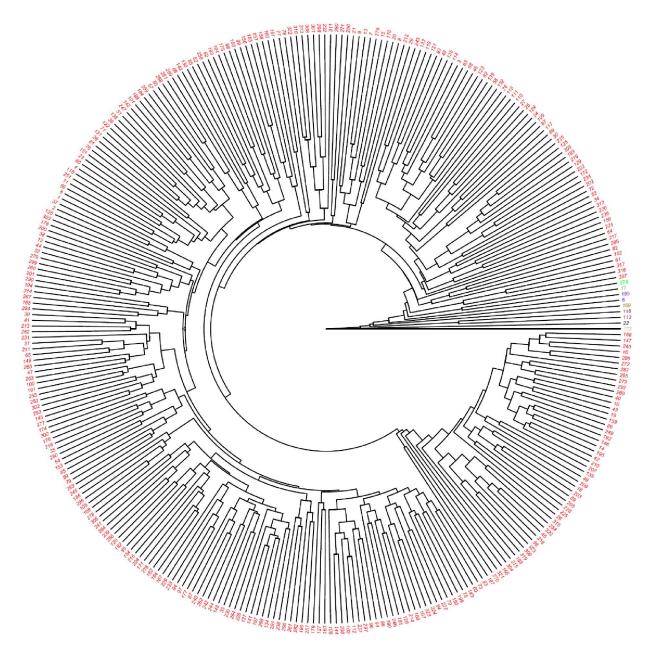


Fig. 1. Grouping of 323 accessions of *C. canephora* by the UPGMA method, based on the Standardized Average Euclidean Distance matrix and 38 characters evaluated in the Germplasm Active Bank, Experimental Farm of Bananal do Norte, Incaper, Cachoeiro do Itapemirim, Espírito Santo, Brazil.

Table 2. Grouping of 323 accessions of *C. canephora* by the Tocher optimization method, based on the Standardized Average Euclidean Distance matrix and 38 characters evaluated in the Germplasm Active Bank, Experimental Farm of Bananal do Norte, Incaper.

Group	N ¹	Accessions
1	251	1, 3, 4, 5, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 23, 25, 26, 31, 32, 33, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 54, 56, 57, 58, 59, 60, 63, 65, 66, 68, 70, 71, 73, 74, 75, 76, 79, 80, 83, 84, 85, 86, 87, 88, 89, 90, 91, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 104, 105, 106, 107, 108, 112, 114, 117, 118, 120, 121, 122, 125, 126, 127, 128, 131, 134, 135, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 151, 152, 153, 154, 155, 156, 157, 159, 161, 162, 163, 164, 165, 167, 168, 170, 171, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 218, 219, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 235, 236, 237, 238, 239, 240, 241, 242, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 267, 268, 269, 271, 272, 273, 275, 276, 277, 278, 279, 281, 282, 283, 284, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 305, 306, 313, 314, 315, 318, 319, 320, 322, 323, 323
2	19	24, 27, 53, 62, 67, 109, 110, 111, 119, 123, 124, 130, 132, 133, 172, 185, 222, 234, 243
3	5	304, 307, 309, 316, 317
4	6	2, 28, 30, 69, 81, 136
5	4	55, 92, 220, 312
6	3	72, 129, 310
7	3	158, 160, 166
8	4	7, 82, 150, 285
9	4	8, 9, 21, 103,
10	3	17, 270, 274
11	3	266, 280, 303
12	2	113, 116
13	2	308, 311
14	2	6, 29
15	2	34, 64
16	1	78
17	1	115
18	1	221
19	1	61
20	1	217
21	1	169
22	1	77
23	1	321
24	1	173
25	1	22

¹ The number of accessions in the subgroup.

Ferrao *et al.* (2005, 2009), in analysis using molecular markers of the RAPD type, found a high divergence between 49 studied genotypes. The average genetic distance for the different combinations was 0.275 (± 0.001), the largest being 0.39382. Using the Tocher grouping method and applying 13 agronomic characters, Ferrao *et al.*, (2017a) with a study on the genetic variability and agronomic performance of 101 hybrid progenies and six parents classified 25 groups and showed important genetic variability for different characters and favorable condition for selection. According to the aforementioned authors, in the definition of the progenies to be grouped to form a new clonal hybrid cultivar,

attention must be paid to the issue of species self-incompatibility, and it is necessary to select genetically different and compatible materials to ensure efficient pollination, adequate fruiting and production.

Conclusion

Based on a set of 38 characters related to plant architecture, production, and fruit maturation, there is an important genetic variability among the 323 accessions of the Active Germplasm Bank of *C. canephora* of Incaper (BAG), evaluated at 24 months in the south of the State of Espírito Santo.

Table 3. The relative contribution of the 38 characters to the analysis of phenotypic variability and averages of the 25 groups obtained in the Tocher grouping, referring to the 323 accessions assessed by the Germoplasm Active Bank at the Experimental Farm of Bananal do Norte, Incaper, Cachoeiro do Itapemirim, Espírito Santo, Brazil.

Characteristics 1	Contribution ²	Groups													
		1	2	3	4	5	6	7	8	9	10	11	12	13	
NR (unit)	0.0478	10.59	7.47	8.60	22.00	7.25	6.67	6.33	10.50	18.25	31.00	6.33	11.00	13.00	
ROL (unit)	0.0462	6.75	3.79	5.00	18.00	3.75	3.00	3.67	6.75	14.25	27.00	2.33	7.00	9.00	
MERO (cm)	10.885	84.98	116.42	102.00	97.67	120.75	23.67	90.00	112.00	116.00	50.67	77.67	130.50	67.50	
MARO (cm)	0.5226	110.68	133.79	121.80	118.50	132.00	136.33	107.67	137.00	122.00	72.00	109.00	139.50	126.5	
DBC (mm)	0.2788	49.10	53.10	53.35	70.77	62.10	62.15	44.66	54.90	53.50	45.72	39.70	61.08	45.87	
NMERO (unit)	0.0868	15.45	21.00	10.60	13.17	18.50	1.67	13.67	18.75	28.00	19.67	18.33	24.50	5.00	
NMARO (unit)	0.0893	22.87	27.11	16.20	24.00	19.25	24.67	36.67	33.50	46.50	29.00	25.67	23.00	20.00	
NRP (unit)	13.538	113.03	150.89	88.40	147.17	121.00	103.33	86.67	129.25	137.75	118.33	106.33	189.00	73.00	
CRPI (cm)	0.286	54.01	70.39	75.00	56.67	76.50	66.33	58.00	47.75	63.50	40.67	46.33	54.00	79.00	
CRPM (cm)	0.191	52.83	67.37	59.00	57.25	67.25	66.67	51.00	54.75	57.00	37.67	58.33	56.00	87.50	
CRPS(cm)	0.1828	30.15	37.16	27.60	28.33	46.50	43.67	22.00	47.00	37.75	24.00	52.33	25.00	56.50	
NRPI (unit)	0.0524	13.97	17.58	19.00	19.83	16.75	11.00	18.00	16.50	13.75	14.33	9.67	13.00	13.50	
NRPM (unit)	0.0213	14.39	17.11	11.00	16.17	13.50	15.67	13.33	22.50	13.75	15.33	16.67	13.00	17.50	
NRPS(unit)	0.0168	7.92	8.95	4.00	9.00	8.75	10.00	6.00	19.50	9.25	7.67	14.33	6.50	10.00	
NFPI (unit)	0.2207	17.33	19.47	40.00	29.00	14.00	16.00	11.67	19.00	11.25	11.00	21.33	8.00	23.50	
NFPM (unit)	0.1509	17.70	22.84	20.00	24.00	27.00	14.67	13.67	18.00	19.25	15.00	14.67	26.50	55.00	
NFPS (unit)	0.0485	13.12	16.00	8.60	19.33	15.50	18.00	12.67	31.00	17.75	13.33	16.67	11.50	14.00	
DC (cm)	0.7579	134.38	157.74	146.40	128.83	168.25	162.00	108.00	137.00	161.25	143.67	135.00	134.00	156.0	
CEMERO (cm)	0.0076	6.14	5.97	10.82	8.23	7.00	13.50	6.68	6.44	4.24	3.05	4.87	5.39	13.45	
CEMARO (cm)	0.0038	5.17	5.27	8.07	5.01	7.18	5.53	3.20	5.40	3.08	2.67	4.34	6.07	6.66	
CERPI (cm)	0.004	4.13	4.05	4.60	2.90	4.70	6.49	3.37	3.15	4.93	2.90	4.60	4.15	6.06	
CERPM (cm)	0.0011	3.81	3.97	5.40	3.58	5.17	4.24	3.81	2.49	4.26	2.67	3.42	4.25	5.00	
CERPS (cm)	0.0024	3.96	4.37	8.05	3.17	5.45	4.43	3.65	2.76	3.98	3.14	3.70	3.93	5.65	
MFROL (g)	714.583	190.09	163.76	245.00	274.17	130.75	63.50	169.33	95.50	622.88	760.33	161.33	1610.00	493.0	
MSROL (g)	11.185	68.75	62.08	98.60	85.25	41.63	23.33	70.50	28.88	211.75	267.50	59.50	611.50	214.7	
NROPI (unit)	0.0213	3.92	9.26	1.40	12.00	5.75	2.67	3.33	2.00	4.50	2.67	0.00	7.00	2.00	
NROPM (unit)	0.0108	2.16	6.89	0.00	4.50	2.75	4.67	0.00	2.25	3.50	0.00	3.67	2.00	0.00	
NROPS (unit)	0.0023	0.12	0.16	0.00	0.00	0.00	0.33	7.33	0.25	0.00	0.00	0.33	0.00	0.00	
NGMARO (unit)	27.074	27.24	102.42	0.80	22.33	13.25	20.33	47.00	17.75	49.75	37.33	50.67	101.50	0.00	
NGMERO (unit)	0.9525	9.38	38.37	0.00	6.17	22.50	0.00	0.00	4.25	27.00	8.00	0.00	96.00	4.00	
NGRPI (unit)	47.329	35.99	126.95	0.00	57.00	61.50	35.67	66.00	23.75	39.75	7.00	0.00	111.00	54.50	
NGRPM (unit)	16.952	18.43	73.37	0.00	40.67	35.25	66.00	36.67	11.00	63.00	0.00	12.33	40.00	0.00	
NGRPS (unit)	0.0145	0.08	0.58	0.00	0.00	0.00	9.00	0.00	1.00	0.00	0.00	0.67	0.00	0.00	
V (%)	0.3005	19.31	23.79	0.00	41.50	38.00	43.67	14.33	12.75	24.25	10.67	3.00	38.00	51.00	
M (%)	0.7315	37.24	51.95	0.00	47.17	39.00	44.67	15.33	71.50	45.00	36.33	13.33	50.00	33.00	
S (%)	0.5608	25.12	24.26	0.00	11.33	23.00	11.67	37.00	15.75	30.75	19.67	83.67	12.00	16.00	
Peso (Kg)	0.0025	1.14	3.95	0.00	1.73	2.14	2.94	0.90	1.32	1.80	0.53	0.94	3.60	1.39	
Boia (%)	0.1636	7.68	7.53	0.00	1.83	43.50	6.67	21.00	1.32	35.00	10.33	13.33	3.50	24.50	

Table 3 (continued)

Characteristics ¹	Contribution ²	Groups											
		14	15	16	17	18	19	20	21	22	23	24	25
NR (unit)	0.0478	8.50	12.50	7.00	4.00	9.00	8.00	17.00	4.00	17.00	11.00	2.00	10.00
ROL (unit)	0.0462	4.50	8.50	3.00	0.00	3.00	4.00	13.00	2.00	13.00	7.00	0.00	6.00
MERO (cm)	1.0885	101.50	101.00	100.00	150.00	117.00	11.00	88.00	139.00	90.00	63.00	106.00	95.00
MARO (cm)	0.5226	122.50	132.25	105.00	155.00	225.00	105.00	105.00	148.00	111.00	104.00	138.00	107.00
DBC (mm)	0.2788	57.23	60.55	54.00	56.47	58.71	55.00	56.64	38.03	50.66	39.84	33.54	43.48
NMERO (unit)	0.0868	53.00	15.00	16.00	24.00	14.00	25.00	11.00	32.00	11.00	8.00	14.00	19.00
NMARO (unit)	0.0893	50.00	22.50	14.00	15.00	19.00	27.00	19.00	19.00	16.00	23.00	35.00	28.00
NRP (unit)	1.3538	145.00	124.00	68.00	162.00	111.00	130.00	124.00	89.00	132.00	71.00	80.00	100.0
CRPI (cm)	0.286	60.00	78.50	16.00	62.00	65.00	21.50	18.00	80.00	50.00	52.00	73.00	56.00
CRPM (cm)	0.191	57.50	62.50	48.00	51.00	62.00	61.00	45.00	67.00	45.00	66.00	92.00	62.00
CRPS(cm)	0.1828	42.50	30.50	20.00	32.00	38.00	67.50	45.00	30.00	22.00	47.00	51.00	66.00
NRPI (unit)	0.0524	14.00	33.50	53.00	16.00	17.00	7.00	14.00	19.00	25.00	2.00	12.00	9.00
NRPM (unit)	0.0213	13.50	12.50	10.00	11.00	10.00	18.00	10.00	12.00	38.00	19.00	19.00	28.00
NRPS(unit)	0.0168	12.50	8.00	6.00	6.00	5.00	17.00	3.00	6.00	8.00	12.00	11.00	16.00
NFPI (unit)	0.2207	13.50	17.00	22.00	11.00	8.00	13.00	11.00	9.00	44.00	3.00	0.00	15.00
NFPM (unit)	0.1509	18.00	19.00	36.00	16.00	13.00	30.00	8.00	23.00	72.00	13.00	0.00	39.00
NFPS (unit)	0.0485	22.00	16.00	10.00	12.00	10.00	27.00	5.00	14.00	9.00	17.00	22.00	30.00
DC (cm)	0.7579	148.50	163.50	108.00	182.00	144.00	180.00	146.00	146.00	148.00	130.00	137.00	175.0
CEMERO (cm)	0.0076	1.95	7.35	6.25	6.25	8.36	0.44	8.00	4.34	8.18	7.88	7.57	5.00
CEMARO (cm)	0.0038	2.46	5.87	7.50	10.33	11.84	3.89	5.53	7.79	6.94	4.52	3.94	3.82
CERPI (cm)	0.004	4.80	2.93	0.30	3.88	3.82	3.07	1.29	4.21	2.00	26.00	6.08	6.22
CERPM (cm)	0.0010	4.31	5.30	4.80	4.64	6.20	3.39	4.50	5.58	1.18	3.47	4.84	2.21
CERPS (cm)	0.0024	3.40	3.81	3.33	5.33	7.60	3.97	15.00	5.00	2.75	3.92	4.64	4.13
MFROL (g)	71.4583	81.75	142.50	515.00	0.00	556.50	138.50	195.00	450.00	631.00	233.00	0.00	26.50
MSROL (g)	11.185	23.75	40.25	208.00	0.00	190.50	53.00	58.00	170.00	192.50	80.00	0.00	8.00
NROPI (unit)	0.0213	8.00	8.00	3.00	12.00	7.00	7.00	7.00	0.00	1.00	0.00	12.00	0.00
NROPM (unit)	0.0108	1.50	4.50	0.00	6.00	0.00	3.00	0.00	0.00	0.00	4.00	12.00	10.00
NROPS (unit)	0.0023	1.00	0.00	0.00	0.00	0.00	0.00	0.00	10.00	0.00	0.00	0.00	7.00
NGMARO (unit)	2.7074	54.00	114.00	0.00	7.00	91.00	38.00	6.00	5.00	0.00	0.00	244.00	71.00
NGMERO (unit)	0.9525	101.50	0.00	8.00	148.00	0.00	72.00	0.00	153.00	0.00	0.00	0.00	0.00
NGRPI (unit)	4.7329	55.00	85.00	8.00	265.00	113.00	48.00	44.00	154.00	0.00	0.00	571.00	0.00
NGRPM (unit)	1.6952	20.50	44.50	0.00	93.00	0.00	35.00	0.00	0.00	0.00	0.00	317.00	95.00
NGRPS (unit)	0.0145	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	44.00
V (%)	0.3005	17.00	26.00	17.00	26.00	7.00	27.00	24.00	17.00	16.00	0.00	22.00	32.00
M (%)	0.7315	58.50	36.00	21.00	64.00	68.00	57.00	15.00	57.00	42.00	0.00	55.00	30.00
S (%)	0.5608	24.50	38.00	62.00	10.00	25.00	16.00	61.00	26.00	42.00	0.00	23.00	38.00
Weight (Kg)	0.0025	1.53	2.97	0.23	4.10	1.68	0.62	1.10	3.73	0.33	0.00	4.50	1.73
Float (%)	0.1636	1.00	2.50	3.00	0.00	3.00	1.00	0.00	17.00	0.00	0.00	4.00	7.00

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Table 3 (continued)

Number of orthotropic branches (NR) (unit), orthotropic branches thrown by plants susceptible to pruning (ROL) (unit), length of the smallest orthotropic branch (MERO) (cm), length of the largest orthotropic branch (MARO) (cm), stem base diameter (DBC) (mm), number of nodes in MERO (NMERO) (unit), number of nodes in MARO (NMARO) (unit), number of plagiotropic branches in the plant (NRP) (unit), length of the lower plagiotropic branch (CRPI) (cm) medium (CRPM) (cm) and upper (CRPS) (cm), number of nodes in the lower (NRPI) medium (NRPM) and upper (NRPS) plagiotropic branches (unit), number of leaves released in the lower (NFPI) (unit), medium (NFPM) (unit) and upper (NFPS) (unit) plagiotropic branches, larger diameter of the coffee tree crown in projection towards the planting line (DC) (cm), length of internodes of the smallest orthotropic branch, MERO (CEMERO) (cm), length of internodes of the largest orthotropic branch, MARO (CEMARO) (cm), length of internodes in the lower plagiotropic branch (CERPI) (cm), medium (CERPM) (cm) and upper (CERPS) (cm), fresh matter ROL (MFROL) (g), dry matter ROL (MSROL), number of rosettes in the lower plagiotropic branch (NROPI) (unit), medium (NROPM) (unit), upper (NROPS) (unit), number of grains in the largest (NGMARO) (unit) and smallest (NGMERO) (unit) orthotropic branch, number of grains per rosette in the lower plagiotropic branch (NGRPI) (unit), medium (NGRPM) (unit), upper (NGRPS) (unit), percentage of green (V), ripe (M) and dry (S) grains based on a random sampling of 100 grains from the plant; Weight of coffee harvested per plant (Weight) (Kg); percentage of grain floats based on a sample of 100 ripe grains per plant (Float).

² Relative contribution of characters to the analysis of genetic divergence given in % based on the methodology proposed by Singh (1981).

The data shows the absence of duplicates in the BAG and there are heterotic groups and distinct accessions in the BAG, which can be inserted in hybridization programs or *per se* to obtain new cultivars. The pairs of most similar and dissimilar accessions were 45 (148/86) and 320 (IAC37) with a statistical distance of 0.0713 and, 173 (ES 1-B) and 270 (403-Marilândia) with a distance of 0.4765, respectively. It is worth noting that genotype 173 also showed the greatest distances to all other studied accessions of the BAG.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

References

- Alkimim ER, Caixeta ET, Sousa TV, Silva FL, Sakiyama NS, Zambolim L. 2018. High-throughput targeted genotyping using next-generation sequencing applied in Coffea canephora breeding. *Euphytica* 214:50 doi:10.1007/s10681-018-2126-2.
- Anagbogu CF, Bhattacharjee R, Ilori C, Tongyoo P, Dada KE, Muyiwa AA, Gepts P, Beckles DM. 2019. Genetic diversity and reclassification of coffee (*Coffea canephora* Pierre ex A. Froehner) from South Western Nigeria through genotyping-by-sequencing-single nucleotide polymorphism analysis. *Genet Resour Crop Ev* 66: 685-696.
- Archana RS, Sudha Rani M, Vishnu Vardhan KM, Fareeda G. 2018. Genetic diversity studies among rice (*Oryza sativa* L.) genotypes for grain yield, yield components and nutritional traits in rice. *Int J Chem Studies* 6: 134-137.
- Berthaud, J. 1986. Les resources génétique pour l'amélioration des caféiers africains diploides. Evaluation de la richesse génétique des populations sylvestres et de ses mécanismes organisateurs. Conséquences pour l'application. University of Paris, Pais, Orstrom.

- Bramel P, Krishnan S, Horna D, Lainoff B, Montagnon C .2017. Global conservation strategy for coffee genetic resources. Crop Trust, World Coffee Res., Portland, *OR*. Available at: https://worldcoffeeresearch.org/documents/42 /Coffee Strategy Low Res.pdf.
- Carmona PAO, Peixoto JR, Amaro GB, Mendonça M. 2015. Genetic divergence of sweet potato accessionsions based on morpho-agronomic descriptors of the roots. *Hortic Bras* 33: 241-250.
- Cecon PR, Silva FF, Ferreira A, Ferrao RG, Carneiro APS, Detmann E, Faria PN, Morais TSS. 2008. Análise de medidas repetidas na avaliação de clones de café 'Conilon'. *Pesq Agropec Bras.* 43: 1171-1176.
- Companhia Nacional de Abastecimento. 2020. Acampamento da safra brasileira: *café*. v. 6, n. 1, p. 1-62, Brasília: Conab, jan.
- Cosme S, Cuevas HE, Zhang D, Oleksyk TK, Irish BM. 2016. Genetic diversity of naturalized cacao (*Theobroma cacao* L.) in Puerto Rico. *Tree Genet Genomes* 12: 1-13.
- Covre AM, Canal L, Partelli FL, Alexandre RS, Ferreira A, Vieira HD. 2016. Development of clonal seedlings of promising conilon coffee (*Coffea canephora*) genotypes. *Aust J Crop Sci* 10: 385-392.
- Cruz CD, Regazzi AJ, Carneiro PCS. 2012. Modelos biométricos aplicados ao melhoramento genético. Viçosa: Ed. da UFV, 514p.
- Cruz CD. 2013. Genes: a software package for analysis in experimental statistics and quantitative genetics. *Acta Sci Agron* 35: 271-276.
- Cruz CD. 2016. Genes Software -extended and integrated with the R, Matlab and Selegen. *Acta Sci Agron* 38: 547-552.
- Cubry P, Bellis F, Pot D, Musoli P, Leroy T. 2013. Global analysis of *Coffea canephora* Pierre ex Froehner (Rubiaceae) from the Guineo-Congolese region reveals impacts from climatic refuges and migration effects. *Genet Resour Crop Ev* 60: 483-501.
- Dalcomo JM, Vieira HD, Ferreira A, Lima WL, Ferrao RG, Fonseca AFA, Ferrao MAG, Partelli FL. 2015. Evaluation of genetic divergence among clones of conilon coffee

- after scheduled cycle pruning. *Genet Mol Res* 14: 15417-15426.
- Davis AP, Chadburn H, Moat J, Sullivan RO, Hargreaves S, Lughadha EN. 2019. High extinction risk for wild coffee species and implications for coffee sector sustainability. *Sci Adv* 5: 1-9.
- Davis AP, Govaerts R, Bridson DM, Stoffelen P. 2006. An annotated taxonomic conspectus of the genus Coffea (Rubiaceae). *Bot J Linn Soc* 152: 465-512.
- Davis AP, Tosh J, Ruch N, Fay MF. 2011. Growing coffee: Psilanthus (Rubiaceae) subsumed on the basis of plastid and nuclear DNA sequences; implications for the size, morphology, distribution and evolutionary history of Coffea. *Bot J Linn Soc* 167: 357-377.
- Denoeud F, Carretero-Paulet L, Dereeper A, Droc G, Guyot R, Pietrella M, Zheng C, Alberti A, Anthony F, Aprea G, Lashermes P. 2014. The coffee genome provides insight into the convergent evolution of caffeine biosynthesis. *Science* 345: 1181-1184.
- Falconer DS. 1981. Introdução à Genética Quantitativa (Tradução de Silva, MA e Silva, JC). *Universidade Federal de Viçosa, Viçosa, MG*, p.279
- Ferrao MAG, Fonseca AFA, Ferrao RG, Barbosa WM, Souza EMR. 2009. Genetic divergence in conilon coffee revealed by RAPD markers. *Crop Breed Appl Biot* 9: 67-74.
- Ferrao MAG, Ferrao RG, Fonseca AFA, Verdim Filho AC, Volpi PS. 2015. Origem, dispersão geográfica, taxonomia e diversidade genética de *Coffea canephora*. *Café conilon*. *Vitória*: *Incaper*, 66-91.
- Ferrao MAG, Fonseca AFA, Ferrao RG, Volpi PS, Verdin Filho AC, Riva-Souza EM, Comerio M, Kaulz M. 2017b. Variabilidade genética de progênies híbridas de *Coffea canephora*. In: congresso brasileiro de melhoramento de plantas, 9., 2017, Foz do Iguaçu. Melhoramento de plantas: projetando o futuro. Foz do Iguaçu: SBMP, 2017.
- Ferrao MAG, Ferrao RG, Fonseca AFA, Verdim Filho AC, Volpi PS. 2019. Origin, geographical dispersion, taxonomy and genetic diversity of *Coffea canephora*. In: Ferrão RG, Fonseca AFA, Ferrão MAG, De Muner LH, eds. Café Conilon, segunda

- edição atualizada e ampliada. Vitória: DCM/Incaper Press, 85-109.
- Ferrao MAG, Fonseca AFA, Barbosa WM, Ferrao RG. 2005. Variabilidade genética em Coffea canephora com base em marcadores RAPD. In: Embrapa Café-Artigo em anais de congresso (ALICE) 2005. in: simpósio de pesquisa dos cafés do brasil, 4., 2005, Londrina. Anais... Brasília, DF: Embrapa Café, 2005.
- Ferrao RG, Fonseca AFA, Ferrao MAG, Muner LH. 2017b. Café Conilon, segunda ediçao atualizada e ampliada. Vitória: DCM/Incaper, 784p.
- Fonseca AFA. 1999. Análises biométricas em café conilon (*Coffea canephora* Pierre).
- Fonseca AFA, Sediyama T, Cruz CD, Sakiyama NS, Ferrao MAG, Ferrao RG, Bragança SM. 2006. Divergência genética em café conilon. *Pesqu. Agropecu. Bras. Pesquisa Agropecuária Brasileira* 41: 599-605.
- International Coffee Organization (ICO). 2018. Trade Statistics. Available at: www.ico.org/trade statistics.asp.
- Laliberté B, Cryer NC, Daymond AJ, End MJ, Engels JM, Eskes A, Gilmour M, Lachenaud P, Phillips-Mora W, Turnbull CJ, Umaharan P. 2012. A global strategy for the conservation and use of cacao genetic resources, as the foundation for a sustainable cocoa economy.
- Lebot V, Melteras M, Pilecki A, Labouisse JP. 2020. Chemometric evaluation of cocoa (*Theobroma cacao* L.) and coffee (*Coffea* spp.) germplasm using HPTLC. *Genet Resour Crop Ev* 67: 895-911.
- Marraccini P, Vinecky F, Alves GSC, Ramos HJO. 2012. Differentially expressed genes and proteins upon drought acclimation in tolerant and sensitive genotypes of *Coffea canephora*. *J Exp Bot* 63: 4191-4212.
- Montagnon C, Leroy T, Yapo AB. 1992. Diversité génotypique et phénotypique de quelques groupes de caféiers (*Coffea canephora* Pierre) em collection. Conséquences sur leur utilisation em sélection. *Café CacaoThé* 36: 187-198.
- Musoli P, Cubry P, Aluka P, Billot C, Dufour M, Bellis F, Pot D, Bieysse D, Charrier A, Leroy T. 2009. Genetic differentiation of wild and

- cultivated populations: diversity of *Coffea* canephora Pierre in Uganda. Genome 52: 634-646.
- Prezotti LC, Oliveira JA, Gomes JA, Dadalto GG. 2013. Manual de recomendação de calagem e adubação para o Estado do Espírito Santo: 5ª aproximação.
- Team Rc. 2019. R: A language and environment for statistical computing v. 3.5. 1. Vienna, Austria: foundation for statistical computing.
- Rabbani MA, Iwabuchi A, Murakami Y, Suzuki T, Takayangi K. 1998. Phenotypic variation and the relationship among mustard (*Brassica juncea* L.) germplasm from Pakistan. *Euphytica* 101: 357-366.
- Rahman MS, Islam SMS. 2020. Genetic diversity analysis based on morphological characters in mulberry (*Morus spp.*) *J Biosci* 28: 111-119.
- RAO RC. 1952. Advanced statistical methods in biometric research.
- Rocha RB, Santos DV, Ramalho AR, Teixeira AL. 2014. Characterization and use of genetic variability of bank active germplasm *Coffea canephora* Pierre ex Froehner. *Coffee Sci* 8: 478-485.
- Silva FL, Baffa DCF, Rezende JC, Oliveira ACB, Pereira AA, Cruz CD. 2015. Genetic variability among robusta coffee genotypes in the state of Minas Gerais. *Coffee Sci* 10: 20-27.

- Silvestrini M, Maluf MP, Silvarolla MB, Guerreiro-Filho O, Medina-Filho HP, Vanini MMT, Oliveira AS, Gaspari-Pezzopane C, Fazuoli LC. 2008. Genetic diversity of a Coffea germplasm collection assessed by RAPD markers. *Genet Resour Crop Ev* 55: 901-910.
- Singh D. 1981. The relative importance of characters affecting genetic divergence. *Indian J Genet Pl Br* 41: 237-245.
- Solórzano RGL, Bellis F, Leroy T, Plaza L, Guerrero H, Subia C, Calderón D, Fernández F, Garzón I, Lopez D, Vera D. 2017. Revealing the diversity of introduced *Coffea canephora* germplasm in ecuador: towards a national strategy to improve robusta. *Sci World J* 2017: 1-12.
- Souza FF, Caixeta ET, Ferrao LFV, Pena GF, Sakiyama NS, Zambolim EM, Zambolim L, Cruz CD. 2013. Molecular diversity in *Coffea canephora* germplasm conserved and cultivated in Brazil. *Crop Breed Appl Biot* 13: 221-227.
- Souza FF, Queiroz MA, Dias RCS. 2005. Divergência genética em linhagens de melancia. *Hortic Bras* 23: 179-183.
- USDA 2019. United States department of agriculture. *Production, Supply and Distribuition*. Available at: https://apps.fas.usda. gov/psdonline/app/index.html#/app/download s.