

Original Research Article

Characterization and Diversity of Peppers (*Capsicum spp.*) Genotypes Based on Morphological Traits Using Multivariate Analysis

ABSTRACT

The present work aimed to characterize and evaluate *Capsicum* peppers in the South of the State of Espírito Santo, in order to estimate the variability among the accessions for the Germplasm Bank implementation. For this, 33 genotypes were collected through donations in four municipalities in the region. The genotypes were characterized based on nine quantitative descriptors: number of seeds per fruit (NS), number of fruit locules (NL), number of fruits per plant (NFR), plant height (PH), leaf width (LW), leaf length (LL), fruit length (FRL) and fruit diameter (FRD). Analysis of variance and means grouping by the Scott-Knott test was performed ($P=0.01$). To estimate the diversity, the Tocher method was realized. The GT Biplot analysis was performed with using RStudio program to estimate discrimination, representativeness and correlation of characteristics. It was found a great diversity among the studied genotypes, being able to emphasize the accession Ifes 01 and Ifes 03 as the most divergent ones. The Tocher grouping allowed the formation of seven groups. The biplot analysis presented the formation of three groups of characteristics. The access Ifes 01 stood out for the group of characteristics FRL, NS and FRD. The Ifes 32 accession was highlighted for the PH and DC characteristics group, and the Ifes 20 accession was highlighted for the group formed by the NFR characteristic. It was observed that the characteristics that contributed the most to the divergence of accessions were PH and DC, and the most representative characteristic was DC. So, it's concluded that there is great variability among the genotypes collected, presenting great phenotypic variation for the nine characters studied. In addition, some accessions were promising for plant breeding programs, pointing to gains in the various segments of the peppers market, which shows the importance of the collections of the producers in the South of Espírito Santo.

Keywords: Peppers, Genetic Resources, GT biplot, Genetic Divergence, Germplasm bank.

1. INTRODUCTION

The genus *Capsicum* belongs to the family Solanaceae and has 38 species, including the new described species, such as *C. caatingae*, *C. longidentatum* and *C. eshbaughii* [1], which are grouped into different categories according to the level of exploitation by man, being: domesticated (widely cultivated), semidomesticated (poorly cultivated) and wild (not cultivated commercially) [2]. In the present study, it was observed that, in the absence of a high level of genetic diversity,

Comment [A1]: Semi domesticated

The largest producers of *Capsicum* peppers in natura in the world are China (15 million tons/year), Mexico (2.8 million tons/year) and Turkey (1.9 million tons/year). For dehydrated peppers, the countries that stand out are India (1.3 million tons/year), China (275 thousand tons/year) and Pakistan (164 thousand tons / year) [3]. The cultivation of pepper in Brazil occurs in almost all regions and the main producing states are Minas Gerais, Goiás, São Paulo, Ceará and Rio Grande do Sul.

There is still a great deal of difficulty in estimating exactly the data about the consumption and production of peppers in Brazil, mainly because the consumer market is divided into several segments with different forms of use and consumption [4]. According to Ribeiro et al. [5] (2008), the fruits of *Capsicum* spp. are important sources of three types of antioxidants: vitamins C and E, and carotenoids.

One of the most important segments of the production and marketing of *Capsicum* in Brazil is in the family agriculture sector, where peppers are marketed in local fairs and trades, mainly in their in natura form [6]. Rural communities can contribute to the use and conservation of germplasm adapted to the agricultural ecosystems of the producing communities [7]. The south of Espírito Santo stands out due to the intense activity of family agriculture, which guarantees income to many families. The peppers of the genus are part of the range of products of these families, therefore, there are in these properties the maintenance of the genetic variability of the crop in question, since many producers carry out the exchange of seeds between them.

Because it is considered a genus that has great diversity and relevant economic importance, it is necessary to carry out genotypic and phenotypic diversity studies, since the information regarding local diversity can guarantee the maintenance of the variability held by the producers, avoiding that processes of genetic erosion occur, and consequently affect the food security of these families. In addition, the characterization of diversity allows the implementation of breeding programs for the selection of superior genotypes for the recommendation to producers.

In the light of the presented questions, this work had the objective of collecting peppers of the genus *Capsicum* in the South of the State of Espírito Santo and proceed with the characterization of the 33 accessions obtained with the purpose of estimating the variability existing between these accesses as well as providing information for the implantation of a Germplasm Bank for *Capsicum* in the Instituto Federal do Espírito Santo – Campus de Alegre.

2. MATERIAL AND METHODS

2.1 Experimental design and cultural practices

The research was developed in the sectors of Olericultura and in the Laboratory of Genetics and Molecular Biology of the Instituto Federal do Espírito Santo – Campus de Alegre, located in the Experimental Farm "Caixa D'Água" in Rive, Alegre district (20°45'18"S e 41°27'10"O). According to the international classification of Köppen, the climate of the region is humid tropical and warm with a cold and dry winter and a hot and rainy summer. The average temperature in the region is 23.1°C, and the average annual precipitation is 1341 mm [8].

The fruits of peppers were acquired through donation received from people who planted on rural properties in the south of the State of Espírito Santo, totaling 33 accessions. The collected seeds were sown in polystyrene trays with 128 cells, containing substrate for the

production of seedlings. The transplanting was carried out after two months for the field conditions, in the Sector of Olericultura, of Ifes - Campus de Alegre.

The experiment was conducted in the period from January to May 2018, and the leaves and fruits of the plants for morphoagronomic analysis were obtained during the months of April and May 2018. For the planting, the spacing of 1.0 m between rows and 0.5 m between plants. The experimental design was a randomized complete block design with 33 treatments and four replications, totaling 132 plants. The cultural treatments were the same as those recommended by [9].

2.2 Evaluated traits

The accesses were characterized by highly discriminating pepper specific essential morphoagronomic descriptors, which are available from Bioversity International (Descriptors for *Capsicum*) [10]. It was used nine quantitative descriptors, namely: number of seeds per fruit (NS), number of fruit locules (NL), number of fruits per plant (NFR), plant height (PH), diameter of the crown (DC) leaf length (LF) and leaf width (LW), fruit length (LFR) and fruit diameter (DFR).

2.3 Statistical-genetic analysis

For the analysis of variance, the following model was considered: $Y_{ij} = \mu + g_i + b_j + \varepsilon_{ij}$, where: Y_{ij} : observed value of the i -th genotype in the j -th block; μ : general constant; g_i : fixed effect attributed to the i -th genotype; b_j : effect of block j ; and ε_{ij} : random error associated with observation Y_{ij} .

Afterwards, the statistical method of Scott-Knott [11] at 1% of probability, was used to compare the means of accessions. The genetic divergence among the accessions was determined by the Tocher grouping method, using the generalized distance of Mahalanobis as a measure of dissimilarity. Statistical-genetic analyzes were performed with the help of the Genes program [12].

Then, the Biplot GT analysis proposed by Yan and Rajcan [13] was performed. The model used in the multivariate analysis applied to biplot genotypes x characteristics (GT Biplot) was: $\frac{T_{ij} - \bar{T}_{ij}}{S_j} = \lambda_1 \zeta_{i1} \tau_{j1} + \lambda_2 \zeta_{i2} \tau_{j2} + \varepsilon_{ij}$, where: T_{ij} is the mean value of genotype i for characteristic j ; \bar{T}_{ij} represents the average of all genotypes in characteristic j ; S_j is the standard deviation estimate; λ_1 and λ_2 represent the unique values for PC1 and PC2, respectively; ζ_{i1} and ζ_{i2} represent the PC1 and PC2 scores for the genotype i ; τ_{j1} and τ_{j2} represent the scores of PC1 and PC2 for the characteristic j ; ε_{ij} is the residue of the model associated to the performance of the genotype and the characteristic. To generate the GT Biplot graph [14] the RStudio software [15] - GGEbiplotGUI package [16] was used.

3. RESULTS AND DISCUSSION

In general, the genotypes presented significant genetic variability, showing a significant difference for all the characteristics evaluated, by the Test F ($P = .01$). The coefficients of experimental variation (CV%) presented values between 13.37%, for number of fruit locules, and 45.92%, for number of fruits per plant (Table 1).

Table 1. Analysis of variance for the nine quantitative characters evaluated in the 33 accessions of pepper (*Capsicum* spp.).

FV	DF	Mean Squares								
		NS	NL	NFP	PH	CD	LL	LW	FRL	FRD
Blocks	3	1120.07	0.97	849.50	2129.72	3302.42	199.44	168.72	2.90	0.74
Gen.	32	604.91**	2.27**	3229.69**	1438.89**	1295.35**	10.34**	5.95**	8.09**	4.87**
Residue	96	108.13	0.14	359.97	77.37	113.91	1.46	0.90	0.44	0.45
CV %		29.75	13.37	45.92	21.60	19.23	21.28	17.28	21.96	34.30

Genotypes (Gen); Coefficient of variation (CV%). NS = Number of seeds; NL = number of locules; NFR= number of fruits per plant; PH = plant height; DC = diameter of the crown; LW = leaf width; LL = leaf length; FRL = fruit length; FRD = fruit diameter.

The highest values for the CV derive from the metric amplitude of some characteristics, which was high because they were different species of *Capsicum*, causing great variation, implying high values of the Mean Square of the residue, resulting in higher CV values.

Based on the means test, it was possible to observe different phenotype classes among the studied genotypes. For the fruit length characteristic (FRL), six classes were observed. For the characteristics of fruit diameter (FRD), diameter of the crown (DC), plant height (PH), number of fruits per plant (NFR) and number of locules (NL) were observed five classes. These results show the high genetic variability for these characteristics. But, for the characteristics of seed number per fruit (NS), leaf width (LW) and leaf length (LL), three classes were obtained (Table 1).

Table 2. Means* of the 33 accessions of pepper with respect to nine morphological characters.

Gen.	NS (un)	NL (un)	NFR (un)	PH (cm)	DC (cm)	LL (cm)	LW(cm)	FRL (cm)	FRD (cm)									
Ifes 01	79.02	a	4.44	a	8.91	e	45.66	d	74.74	b	7.70	b	6.25	a	4.16	d	5.10	a
Ifes 02	45.42	b	2.34	d	67.78	c	63.27	c	76.94	b	7.22	b	6.05	a	2.55	e	0.74	e
Ifes 03	41.75	b	3.25	b	43.25	d	43.00	d	59.00	c	7.64	b	7.55	a	2.91	e	1.93	d
Ifes 04	45.50	b	2.00	e	9.00	e	18.38	e	29.75	e	3.34	c	5.57	a	2.58	e	1.15	d
Ifes 05	33.94	b	2.87	c	30.29	d	31.32	e	58.70	c	6.77	b	6.64	a	5.12	c	1.26	d
Ifes 06	23.02	c	1.94	e	34.41	d	89.16	a	73.24	b	6.53	b	6.19	a	2.83	e	0.58	e
Ifes 07	29.50	c	3.25	b	13.75	e	37.25	d	52.00	c	6.13	b	6.18	a	3.36	e	3.58	b
Ifes 08	50.02	b	3.94	a	10.41	e	34.16	d	46.74	d	5.80	b	5.71	a	4.72	c	2.61	c
Ifes 09	42.52	b	3.44	b	41.91	d	48.16	d	59.99	c	6.30	b	5.72	a	1.59	f	2.08	c
Ifes 10	34.25	b	2.50	d	19.50	e	27.00	e	46.50	d	3.72	c	3.40	c	2.18	e	1.84	d
Ifes 11	38.75	b	4.00	a	15.44	e	38.27	d	56.27	c	6.30	b	6.12	a	3.68	d	4.51	a
Ifes 12	30.52	c	2.44	d	17.91	e	18.66	e	32.74	e	4.27	c	4.28	b	1.94	f	1.28	d
Ifes 13	21.70	c	2.29	d	56.70	c	74.21	b	77.57	b	6.49	b	6.51	a	2.71	e	0.59	e
Ifes 14	19.75	c	2.25	d	70.75	c	43.75	d	50.25	c	4.60	c	5.04	b	1.28	f	1.14	d
Ifes 15	39.02	b	2.94	c	32.41	d	25.16	e	66.24	c	5.84	b	6.21	a	5.71	b	1.24	d
Ifes 16	37.25	b	3.50	b	57.50	c	41.00	d	51.25	c	4.72	c	4.74	b	3.02	e	2.30	c
Ifes 17	35.44	b	3.37	b	14.79	e	23.32	e	29.70	e	5.54	b	6.44	a	4.03	d	2.53	c
Ifes 18	23.42	c	4.00	a	31.78	d	23.60	e	43.27	d	6.08	b	5.71	a	2.35	e	1.59	d
Ifes 19	46.52	b	2.94	c	13.91	e	46.66	d	56.99	c	10.74	a	6.32	a	4.99	c	1.12	d
Ifes 20	31.03	c	2.29	d	129.37	a	61.21	c	86.24	a	6.41	b	6.88	a	1.91	f	0.49	e
Ifes 21	21.09	c	2.00	e	90.78	b	57.60	c	56.27	c	4.70	c	4.90	b	1.83	f	0.79	e
Ifes 22	33.75	b	3.67	a	40.78	d	39.94	d	73.94	b	4.98	c	5.03	b	2.56	e	2.39	c
Ifes 23	38.67	b	2.94	c	66.21	c	48.22	d	57.76	c	6.08	b	5.98	a	2.87	e	1.67	d
Ifes 24	16.02	c	1.94	e	28.91	d	15.16	e	19.24	e	2.81	c	2.99	c	1.48	f	0.65	e

lfes 25	47.00	b	2.50	d	19.50	e	36.00	d	72.75	b	6.11	b	5.47	a	7.32	a	2.14	c
lfes 26	40.75	b	2.00	e	98.44	b	31.94	e	52.60	c	5.68	b	5.75	a	4.67	c	1.21	d
lfes 27	36.02	b	1.94	e	38.41	d	24.16	e	29.74	e	2.89	c	3.50	c	2.78	e	0.95	e
lfes 28	44.00	b	3.50	b	22.75	e	43.25	d	62.88	c	6.74	b	6.40	a	2.53	e	2.71	c
lfes 29	27.17	c	1.94	e	60.71	c	22.22	e	30.26	e	3.90	c	3.77	c	1.09	f	0.67	e
lfes 30	20.67	c	3.44	b	45.71	d	41.22	d	56.76	c	5.55	b	4.20	b	1.16	f	1.39	d
lfes 31	23.93	c	3.29	b	68.50	c	42.84	d	67.89	b	4.53	c	4.99	b	1.86	f	2.54	c
lfes 32	20.36	c	1.96	e	37.70	d	91.21	a	92.24	a	7.25	b	7.75	a	3.02	e	0.63	e
lfes 33	35.67	b	1.94	e	25.21	e	17.22	e	31.26	e	3.88	c	3.03	c	2.66	e	1.41	d
Means	34.95		2.82		41.32		40.73		55.51		5.67		5.49		3.01		1.72	

* Means followed by the same letter, in each column, belong to a same class, in accordance with the Scott-Knott test ($P=0.1$). NS = Number of seeds; NL = number of locules; NFR= number of fruits per plant; PH = plant height; DC = diameter of crown; LW = leaf width; LL = leaf length; FRL = fruit length; FRD = fruit diameter.

As in the present work, Bianchi et al [17] in a study with 30 accessions of *Capsicum* also found great variability for fruit diameter and plant height characteristics, with formation of seven and eight phenotypic groups, respectively, for these characteristics. As for the number of seeds, leaf width and leaf length, the authors also report lower variability among the genotypes, with three groups for each of the mentioned characters.

For the number of seeds per fruit (NS), the minimum and maximum values were found in the lfes 24 and lfes 01 accessions, with 16 and 79 seeds, respectively, the overall average for this characteristic being approximately 35 seeds per fruit. For the number of locules (NL) the values ranged from 1.94 to 4.44, with the lowest value found in the lfes 06 access and the highest in the lfes 01 access, with an average of 2.82 locules per fruit. As expected, we found lower variation for the genotypes based on this characteristic, corroborating with results found by previous studies [17-18].

The fruit number characteristic (NFR) presented great variation with values between 8.91 and 129.37 fruits per plant, with the lfes 01 access being the least productive and the lfes 20 access which produced the most. The average production of the accesses was 41.32 fruits per plant. For plant height (PH) and diameter of the crown (DC), the lowest values were 15.16 and 19.24 cm, respectively. The mean values of the two characteristics were 40.73 cm for AP and 55.51 cm for DC. The lfes 24 access presented the smallest measure for the two characteristics while the lfes 32 access presented the greatest measures for the two characteristics with 91.21 and 92.24cm respectively. These results were similar to those found by Bianchi et al. [17] in which the overall plant genotype mean was 54.29 cm. The great variability of the genotypes for these characteristics (Table 2) is of extreme importance for the market of ornamental peppers, since the height of the plants and the diameter of the crown are factors that influence the good architecture in the vessel [19].

Comment [A2]: Diameters of the crown are

According to Henz and Costa [20] in Brazil there are few ornamental peppers cultivars available in the market, even with the extensive variability existing in the genus. Therefore, the importance of the germplasm bank characterization is emphasized, since the knowledge and use of this variability allows, through genetic combinations, the emergence of new and more promising cultivars for the ornamental trade segment [21]. In addition, in future works of multiplication and regeneration of accesses, the knowledge of these characteristics becomes essential for the definition of the experimental design, to define the most adequate spacing between the plants [17, 22].

For the characteristics of width (LW) and leaf length (LL), the averages found were 5.67 and 5.49 cm, respectively. The width ranged from 10.74 cm (lfes19) to 2.81 cm (lfes24), while the length ranged from 7.55 cm (lfes 03) to 2.99 cm (lfes 24). Bianchi et al. [17] found, for

leaf width, measures ranging from 2.23 cm and 6.71 cm. As for leaf length, the authors reported values between 3.00 cm and 10.26 cm.

The values found for fruit length in this work were partially similar to the results observed by Domenico et al. [23], which evaluated peppers 'de cheiro', 'murupi', 'biquinho', among others. The results found by these authors were from 2.1 to 7.7 cm for the characteristic in question. Small fruits such as those presented by the Ifes 20 and 21 accesses have great commercial potential [22] due to the preference of consumers for this in natura fruit. According to Rêgo et al. [18], in relation to the greater length of the fruits, these have great importance due to its high correlation with other characters of interest such as productivity, standing out for this characteristic the access Ifes 25.

In the study of genetic diversity, the most divergent accessions were Ifes 01 and Ifes 02, with a distance of 121.18, and the nearest ones were Ifes 05 and Ifes 15, with a distance of 3.49. The grouping of the genotypes based on the Tocher grouping method resulted in the formation of seven groups (Figure 1). The cophenetic correlation coefficient (CCC) was 0.73, which shows that the fit of the dissimilarity matrix and the cophenetic matrix can be considered adequate, according to Rohlf [24], guaranteeing reliability in the dendrogram interpretation.

Group 1 grouped four accessions and was characterized by gathering genotypes with the highest leaf width (LW) measurement. The Group II only had access to Ifes 26 (characterized by having a greater measure for fruit length, lower values of plant height and number of locules). Group III had three accessions, characterized by having significantly similar values for number of seeds (NS), leaf width (LW), leaf length (LL), fruit length (FRL) and fruit diameter (FRD). Group IV was represented by four accessions. In this group, the accessions present low values for width (FRL) and fruit diameter (FRD) and high values for leaf width (LW). Group V gathered seven accesses with the lowest numbers of locules (NL) among the others. Bianchi et al. [17] reported the presence of eight clusters based on morphological descriptors using the Tocher method. In a characterization study of 59 accessions based on 15 qualitative descriptors of *Capsicum*, SUDRÉ et al. [25] obtained the formation of eight groups by the same method.

According to Bento et al. [22] the multi-stage variables are more advantageous in the characterization study because they are easy to observe and require less time and labor, and their use in banks and collections of germplasm that do not have many human and financial resources.

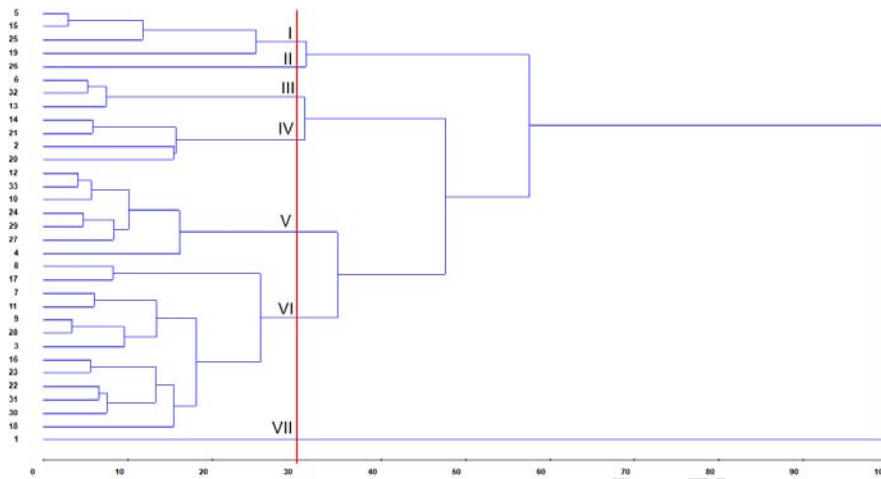


Fig. 1. Dendrogram of genetic dissimilarities obtained by the Tocher method, based on nine quantitative descriptors among 33 accessions of pepper (*Capsicum* spp.)

The analysis of *genotype vs. characteristics* of the GT biplot represented 71.33% of the variation among the studied genotypes. According to Yang et al. [26], the first two main components (PCs) should explain more than 60% of the data variation. The data in this study fulfilled this assumption, suggesting that the graph (Figure 2) efficiently represented the data variation, allowing the safe interpretation of the studied phenomena.

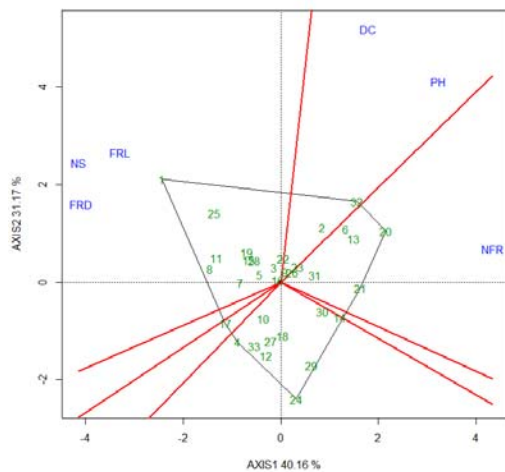


Fig. 2. Analysis of *genotypes vs. treatments* by the Biplot graph "Which-won-Where" for the six characters of commercial importance in *Capsicum* accessions. NS = Number of seeds; NFR= number of fruits per plant; PH = plant height; DC = diameter of the crown; FRL = fruit length; FRD = fruit diameter.

For this analysis only those characteristics of greater economic importance for the culture were used, since the others had little or no relevance for the commercial indication of possible superior genotypes, reducing the number of characteristics for the analysis in question, facilitating the interpretation of the results.

The genotypes that showed the best performance can be observed in the "which-won-where" graph of the biplot, which was divided into groups (Figure 2). These groups are separated by perpendicular lines used to identify the genotypes. Thus, the genotypes located at the vertices are far from the center of origin of the graph, indicating their best performance for the group of characteristics to which they belong [28-29, 16]. In contrast, the genotypes within the polygon are those less responsive to the characteristics studied [16].

The genotype Ifes 01, located at the apex of Biplot (Figure 2), is one of the most promising because it presents the highest values for the fruit length (FRL), number of seeds (NS) and fruit diameter (FRD). The genotype Ifes 32 was highlighted in the group of characteristics formed by plant height (PH) and diameter of the crown (DC), which are essential attributes for ornamental pepper breeding programs, since they contribute significantly to good plant architecture in the vessel [19]. On the other hand, the genotype Ifes 20 stood out for the characteristic number of fruits per plant (NFR), which alone formed only one group.

Based on the graph analysis of the "Discriminative vs. representative" the most discriminatory variables were PH and DC (Figure 3). However, in general, all the characteristics were able to discriminate well the studied genotypes. The most representative characteristics were diameter of the crown (DC) and number of fruits (NFR). Discriminatory and representative characteristics are important information for a breeding program [29]. The ability of a characteristic to discriminate a genotype is highlighted by the size of the vector (Figure 3 - dashed line).

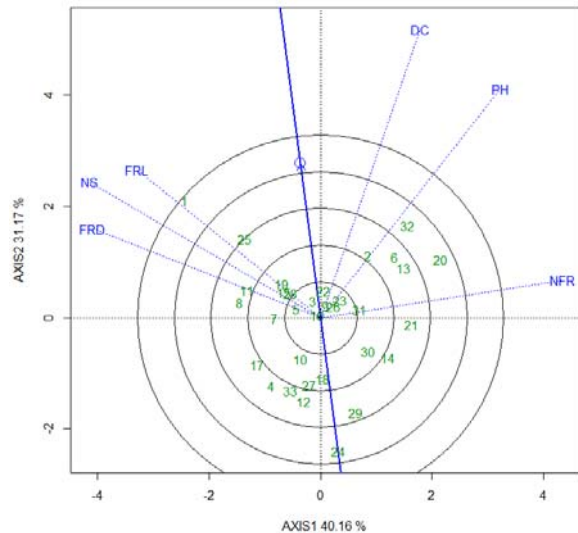


Fig. 3. Analysis of genotypes x traits by Biplot graph "Discriminative x Representative". NS = Number of seeds; NFR= number of fruits per plant; PH = plant height; DC = diameter of the crown; FRL = fruit length; FRD = fruit diameter.

Comment [A3]: Replace x by ×

Comment [A4]: Replace x by ×

The longer the vector, the more discriminant is the characteristic [31]. The most representative variables are those that form the smallest angles with the line that presents the circle formed with the arrow, *i.e.*, with the medium [31]. In addition, the ideal variable should be able to discriminate genotypes and represent the other characteristics, thus, it should present high CP1 (greater discriminatory capacity) and low CP2 (greater representativity) [28, 29] Studies with *Capsicum* using biplot analysis are not yet described in the literature, however, recent studies have reported success in discriminating promising genotypes in other cultures, based on this type of graph, with PC values ranging from 60 to 80% [29, 32].

In biplot analysis, the angle formed between the vectors (dashed lines) of two characteristics shows information about its correlation coefficient. This correlation may be positive, negative or non-existent if the angles are sharp, obtuse or straight, respectively. In a breeding program, the study of the correlations between the characteristics is of great importance, since this information allows the best selection for the characteristics of interest [33]. In this work, we have shown that the correlation between the characteristics of the soil and the soil is more frequent in the soil.

There was a strong positive correlation between fruit number characteristics (NFR), number of seeds (NS) and length of fruits (CF), indicating that the selection of genotypes based on any of these characteristics will imply positive gains in the other. There was also a positive correlation between crown diameter (DC) and plant height (AP) (Figure 3).

Therefore, for selection of genotypes in an ornamental pepper breeding program, based on these results, it is known that taller plants tend to have a larger diameter of the crown and vice versa, facilitating the breeder's work in predicting genotype selection results for this branch of the pepper market.

4. CONCLUSION

There is great variability among the genotypes collected based on the great phenotypic variation for the nine characters studied. In addition, some accesses were promising for the plant breeding, pointing to gains in the various segments of the peppers market, which shows the importance of the collections of the producers in the South of Espírito Santo need the implementation of the Germplasm Bank to ensure the maintenance of this variability.

The observed correlation in the attributes of the fruit (length and diameter of fruits and number of seeds per fruit) allows predicting gains in a selection based on any of these characteristics. Fruits traits also contributed the most to genetic diverge among accessions studied, indicating

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