



PARAMETERS AND GENETIC VARIABILITY IN BLACK BEANS F₅ FAMILIES

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Abstract: The objective of this work was to contrast expectations of the segregating generations F₂, F₃, F₄, and F₅ with homozygous genetic constitutions, to determine the genetic parameters, dissimilarities through multivariate models, and the associations between characters of agronomic importance for the breeding of black beans. The experiment was conducted in the 2017/2018 crop season in the county of Tenente Portela – RS, Rio Grande do Sul, Brazil. The experimental design used was that of augmented blocks, with the cultivars IAC Imperador, IPR Tangará, BRS Estilo composing the common treatments, considered as pure lines, and 131 lineages of the F₅ segregating generation, composing the unusual treatments, arranged in three blocks. After the characterization of the 134 genotypes, it was possible to verify that through Singh's relative contribution, the character of number of seeds per plant was the most decisive for the genetic variability of this study. The use of biometric methods allows it to be possible to evidence patterns in relation to the genotype clusters, through the use of standardized average Euclidean distance patterns, Tocher's optimization method, UPGMA clustering method, and Artificial Neural Networks with unsupervised learning. With the results obtained, it was possible to verify the existence of genetic variability among the studied populations, allowing the cluster analysis to delimit crossings between accessions with genetic potential for the formation of superior genotypes.

Keywords: *Phaseolus vulgaris* L., improvement of plants, multivariate selection of traits, food security, sustainable development.

Introduction

Black beans (*Phaseolus vulgaris* L.) is a leguminous of great agricultural importance, standing out mainly for the distribution of production in terms of production chain levels, chemical composition and nutritional value of grains. Beans are a staple food in the diet of the Brazilian population, being a notable protein source, therefore, widely grown throughout the Brazilian territory, whether in small, medium, or large rural properties (Silva and Wander, 2013).

Brazil is the world's largest producer of beans. In the 2017/2018 harvest, approximately 3.1 million tons of grain were produced. Among the regions with the greatest contribution to the amount of production, the southern region stands out with 27.7% of the total produced in the country (Conab, 2018).

Given the importance of crop, it is crucial to develop cultivars that meet the specific needs of each producing region, and the search for technical and scientific information to enhance the growing of this leguminous is pertinent, as well as the development of new technologies especially for the most varied producing micro-regions in Brazil.

Likewise, increases in productivity are sought, in order to improve to the bean production chain. More productive cultivars result from the selection of families that present desirable agronomically characteristics, stable in the face of climatic weather, tolerant to biotic and abiotic stresses, in addition to having high productive ceilings. In view of this, breeders direct the selection of multiple characteristics simultaneously, so that the most distinct characteristics that make up the culture's ideotype can be present, this then made available to the farmer (Carvalho et al., 2018a).

There are many methodologies available to express the components of variance, genetic parameters, genetic distances, and multivariate similarity or dissimilarity profiles. Where, the use of these strategies can guide what will be the best criteria and genotypes to be used in the subsequent segregating generations. The objective of this work was to contrast expectations of the

segregating generations F₂, F₃, F₄, and F₅ with homozygous genetic constitutions, to determine the genetic parameters, dissimilarities through multivariate models, and the associations between characters of agronomic importance for the breeding of black beans.

Material and methods

The experiment was conducted in the 2017/2018 crop season in the county of Tenente Portela - RS, located at 27 ° 23'31.04"S and 53 ° 46'50.71" W, with an altitude of 420 meters. The region's climate is humid subtropical Cfa type according to the Köppen classification (Kottek et al., 2006) and the soil is classified as a typical ferric aluminum red Latosol (Oxisol) (Santos et al., 2018).

The experimental design used was that of augmented blocks (Federer, 1956), with the cultivars IAC Imperador, IPR Tangará, BRS Estilo composing the common treatments, considered as pure lines, and 131 lineages of the F₅ segregating generation, composing the unusual treatments, arranged in three blocks.

The experimental units were composed of three sowing lines with ten meters in length spaced by 0.45 meters. Direct and manual sowing was carried out in the second half of November, using a sowing density of 22 seeds m⁻². For planting fertilization, 250 kg ha⁻¹ of the formulated 10-20-20 was used, and for topdressing at the phenological stage V4, 90 kg ha⁻¹ of nitrogen was applied in the form of urea (46% nitrogen). In order to minimize the biotic effects, preventive control of weeds, insect pests, and diseases was performed.

The characters of agronomic interest measured in 10 random plants were the first pod insertion height measured from the stem insertion to the first viable pod (PI, cm); plant height, measured from the stem insertion to the apex of the plant, (PH, cm); number of seeds per plant obtained by counting the total number of viable seeds in the plant (NS, units); number of pods per plant, measured by counting the total number of viable pods contained in the plant (NP, units); total mass of seeds per plant obtained from the sample of five plants per experimental unit (SM, grams).

The data obtained were submitted to descriptive analysis, where the expectations for each segregating generation and crop seasons of the pure lines were determined. The generations comprised 40 F₂, 69 F₃, 41 F₄, and 134 F₅ populations.

After analysis of variance with 5% probability, linear correlation was performed in order to verify the trend of association between the characters. After the Euclidean algorithm was used to make the matrix of genetic distances of the studied genotypes using all the measured characters, through the distance matrix, the Tocher's optimized grouping method was applied with intra-group and inter-group stratification. Singh's (1981) relative contribution methodology is used in order to define which characters are most important for distinguishing groups. The phenotypic matrix was subjected to the algorithm with unsupervised machine learning where the Artificial Neural Networks were constructed using the Kohonen Map, establishing the centroids of interest and the explanatory synaptic connections (Carvalho et al., 2018b). Statistical analyzes were performed using the Genes software (Cruz, 2013).

Results and discussion

Expectation in segregating generations

In order to understand the mathematical expectation of the characters measured over the segregating generations in comparison to the pure lines (cultivars), the average character standards for the first pod insertion height (PI), plant height (PH), number of pods per plant (NP), number of seeds per plant (NS), and seed mass per plant (SM) were established, where, with these results, it was possible to infer the standard tendencies of the characters over the generations.

The first pod insertion height showed a similar trend in relation to the plant height (Figure 1A). The pure lines showed greater discrepancy between the crop seasons compared to the segregating generation. There was an increase in the first pod insertion height during the segregating generations until F₄, with values close to 20 cm. The first pod insertion combined with the plant height are very important

characters in study and conduction of segregating families of plants (Gonçalves et al., 2016). In general, commercial bean genotypes have a low first pod insertion height, which can compromise mechanized harvest rates and result in low plant height (Arruda et al., 2010). Thus, the increase observed in the segregating generations for the first pod insertion height is an important characteristic for the selection of new cultivars, since it facilitates the management and harvesting practices of the grains (Demari et al., 2015) as well as reducing the losses and negative effects on the physiological quality of seeds (Terasawa et al., 2009).

In relation to the plant height character, the segregating generations showed stability between the crop seasons, showing less pronounced elevation peaks compared to those observed in the behavior of the cultivars (pure lines) (Figure 1B). In the analysis carried out, it should be taken into account that the results of the graphs in question correspond to the treatment means, that is, for the pure lines the means refer to three cultivars and for the segregating generation the means refer to 134 populations of the segregating generation, formed by the selection of the best individuals from previous generations, which results in greater homogeneity of results over the crop seasons. In F₅, segregating generations are more than 15 cm tall, according to a study by Gonçalves et al. (2016), favors mechanized harvesting, providing the reduction of losses and damages in the harvesting process, as well as the reduction of disease infestation, by preventing the pods from being in contact with the soil.

The segregating generations obtained an increase in the F₅ generation in the number of pods per plant in relation to the behavior presented by the cultivars (Figure 1C). This result shows that there was a change in the phenotypic characteristics of the plants, which consequently can result in an increase in grain yield. The number of pods per plant is an impacting factor in grain yield, where the increase in the number of pods per plant results in greater production (Ribeiro et al., 2003; Gonçalves et al., 2003). In a study by Jadoski et al. (2000), the number of pods per plant shows a high plasticity in relation to the

growing of beans, as it has a direct correlation with the growing density. In certain cases, there is a compensation in lower densities with a greater number of pods per plant.

The pure lines obtained a superior result in the character number of seeds per plant in relation to the segregating generation (Figure 1D). This behavior can be justified by the fact that the cultivars currently available on the market have the

characteristic of well-defined productive potential, taking into account that the plants in F5 still have the possibility of changes, given the variances attributed to non-additive genetic actions.

In studies as in Menezes et al. (2016), it is possible to evidence the association of medium to high genetic variability in segregating populations with a probability of generating lines higher than the standard cultivar.

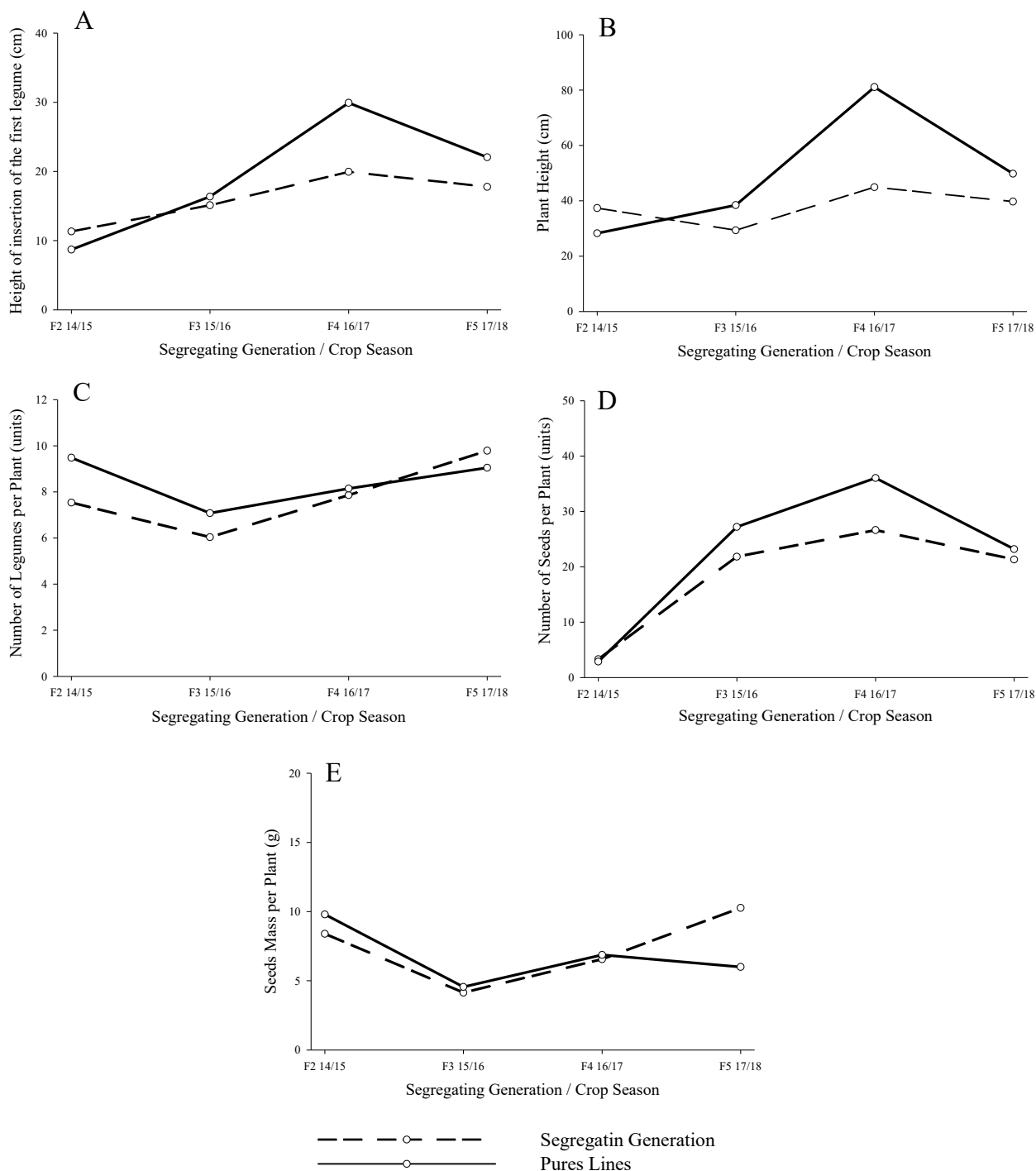


Figure 1. Mathematical expectations for the characters First pod insertion height (A), Plant height (B), Number of pods per plant (C), Number of seeds per plant (D), Seed mass per plant (E), of segregating bean populations and families, conducted in the municipality of Tenente Portela, Brazil, 2019.

The agronomic character seed mass per plant is one of the most relevant for grain yield, whereas the increase in seed mass per plant, automatically, there is a direct interference in the grain yield of the crop. As can be seen in Figure 1E, the demonstration that in the last crop season the segregating generations had superior results in relation to the pure lines, which can be attributed to the selection process carried out over the crop seasons. The breeding process seeks to improve yield components in order to have a higher grain yield. In studies by Coelho et al. (2007), the seed mass per plant was one of the fundamental factors for grain yield and determinant for the separation of bean accessions. Research on bean breeding claims that greater productivity can be achieved by reducing the first pod insertion height, increasing the number of pods per plant and seed mass (Mambrin et al., 2015).

Variance components and genetic parameters

The components first pod insertion height (PI), number of pods per plant (NP) and number of grains per plant (NS) have lower estimates of heritability as a result of a greater effect of the environment on these characters (Table 1).

Table 1. Components of variance and genetic parameters of the character's first pod insertion height (PI), plant height (PH), number of pods per plant (NP), and number of seeds per plant (NS) of segregating bean populations and families conducted in Tenente Portela, Brazil, 2019.

LRT*	Traits			
	PI (- +) 62.68	PH (- +) 373.45	NP (- +) 111.35	NS (- +) 5.41
σ^2_P	30,870	224,744	33,888	58,590
σ^2_G	8,024	144,528	12,368	13,652
σ^2_E	22,846	80,21	21,52	44,938
H^2	0.259	0.643	0.364	0.233
ac	0.509	0.801	0.604	0.482
μ	20,843	49,543	10,099	20,322

*Phenotypic variance (σ^2_P); Genotypic variance (σ^2_G); Environmental variance (σ^2_E); Broad sense heritability (H^2); accuracy (ac); overall mean (μ).

The component that showed the highest genetic variance was the plant height (PH), consequently, this character has a higher

coefficient of heritability, presenting greater potential for selection.

According to Ramalho et al. (1993), it is essential that high heritabilities are considered to achieve superior genotypes. Among the parameters evaluated by Bonnet et al. (2006) studying the genetic divergence in common bean germplasm collected in the state of Paraná, the plant height is among one of the parameters that presented heritability coefficients whose values were greater than 80%. Similar results for the plant height parameter were also found in studies of genetic parameters in bean cultivars by (Ferrão, 202; Coelho et al., 2002; Lana et al., 2015; Peternelli et al., 2015).

Dendrogram

The analysis of the dendrogram performed by the UPGMA Hierarchical method (Figure 2) allowed the formation of seven groups. Similarities were found between the segregating generations, so that the cultivars BRS Estilo, IAC Imperador, and IPR Tangará were present in the large group of 86 accessions. Another large group formed from F61 to F15.

Similar results were verified by Coelho et al. (2010) evaluating genotypes of landrace beans, which were separated into groups with different numbers of representatives, where the vast majority were inserted in a single group and some genotypes were isolated in different groups. Pereira et al. (2019) comment that for a cultivar to be accepted by growers, it must join desirable phenotypes for various characteristics of interest. For this reason, it is important that these traits should be considered throughout the process of developing lines, including assessment and selection of segregating populations.

Tocher's optimization grouping

Tocher's method allows the formation of groups, where the average dissimilarity within each group is adopted as a criterion, being smaller than the average distances between groups (Cruz et al., 2012) Through the evaluated characteristics, this method provided to fragment the genotypes in seven groups (Table 2).

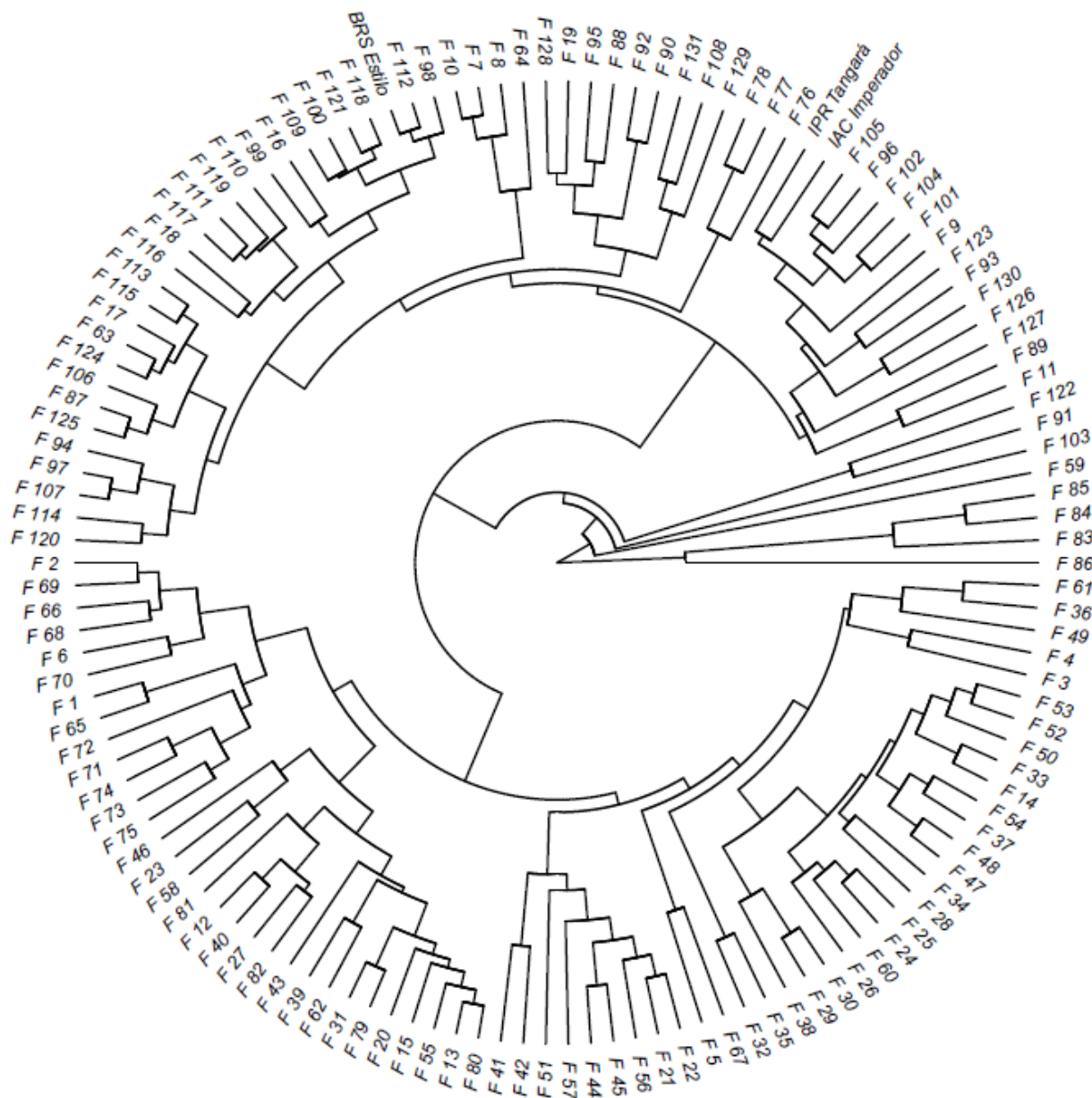


Figure 2. Dendrogram based on the Mean Euclidean distance (UPGMA) of 131 F₅ segregating bean families conducted in Tenente Portela, Brazil, 2019.

Table 2. Tocher's optimization grouping method for the definition of groups through genotype dissimilarity, 131 F₅ segregating families and three commercial cultivars of common black beans, conducted in Tenente Portela, Brazil, 2019.

	Group	Genotypes
Group formation	< 1 >	F112 IPR Tangará F98 F100 F121 F118 F109 F115 F119 F113 F124 F110 F117 F63 F111 F18 F120 F17 F125 F87 F101 F99 F74 F71 F97 F107 F114 F75 F106 F116 F108 F16 F105 F104 F94 F9 IACImperadorF7 F19 F128 F72 F96 F76 F95 F10 F8 F73 F88 F129 F126 F92 F89 F102 F77 F78 F131 F90 F93 F64 F6 F66 F68 F69 F2 F70 F11 F65 F123 F130 F1 BRS Estilo F127 F5 F4 F3 F25 F26 F27 F24 F23 F60 F30 F20 F67 F21 F28
	< 2 >	F13 F80 F55 F15 F31 F79 F39 F82 F43 F12 F40 F62 F53 F52 F54 F50 F33 F37 F48 F47 F14 F44 F81 F34 F45 F56 F36 F61 F38 F29 F22 F58 F57 F46 F32 F49 F51 F41 F42 F35
	< 3 >	F84 F85 F83
	< 4 >	F91 F122
	< 5 >	F59
	< 6 >	F86
	< 7 >	F103

In group 1, the majority of segregating families were arranged, where a total of 86 accessions were collected (63.7%), group 2 presented 41 accessions (30.37%) and from group 5 they presented only 1 accession in each. However, it appears that this method generated a small number of groups, with more than 90% of accessions distributed in only two groups.

With this formation of groups, the crossings that may be more effective for the formation of genotypes are with individuals belonging to different groups, with preference among the most distant groups. Corroborating what Ramalho et al. (1993) in which the most satisfactory results can be obtained through crosses between genotypes of different groups with characteristics of agronomic interest. It is interesting to emphasize that the use of several gene sources can make it possible to obtain superior individuals, due to the genetic complementation between the parents.

Vasconcelos et al. (2007), show that this method has an inconvenience in grouping genotypes, as it is influenced by the distance between the generations already grouped, occurring due to the fact of using a global grouping criterion, based on shorter distances found in the dissimilarity matrix. Similar results were found by studying genetic diversity and indicating crosses in fava beans, and by Gonçalves et al. (2014), in a study of the genetic divergence of common bean accessions through the characteristics of the seed.

In a study by Bertan et al. (2006), similarity between the grouping methods represented by wheat accessions was found. Both in the UPGMA and Tocher method, the results presented are similar to those found in relation to the genetic divergence found.

Relative contribution of Singh (1981)

The contribution of variables to group discrimination by the Singh method is given according to the statistics proposed by Singh (1981). The number of seeds per plant was the character that most contributed to the genetic variability of beans, representing 48.5% of the total (Figure 3).

A study by Grigolo et al. (2018), demonstrates that the characteristics in relation to the seed are responsible for a greater genetic divergence. Cabral et al. (2011), in a study with common beans, demonstrated that the morphological characteristics of the seed are the main sources of variations in genetic divergence. For the other characters, there was a balance in the relative contribution to genetic dissimilarity, without the need to discard any variable for the evaluation.

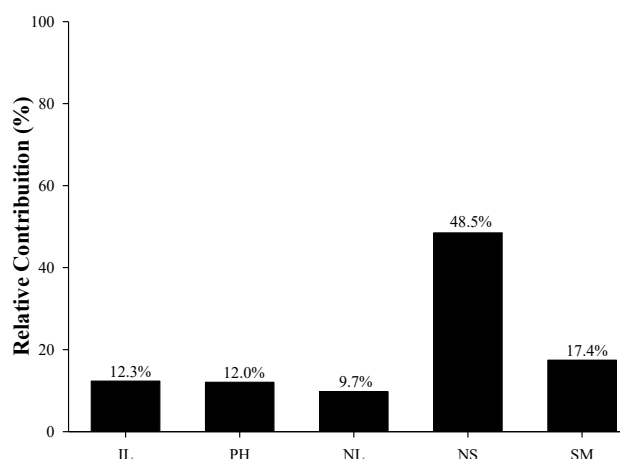


Figure 3. Singh's relative contribution to the characters first pod insertion height (PI), Plant height (PH), Number of pods per plant (NP), Number of seeds per plant (NS), Seed mass per plant (SM) of beans segregating populations and families, conducted in the county of Tenente Portela, Brazil, 2019.

Linear correlation

For the results obtained, it can be observed that there is a significant positive correlation of the number of seeds per plant with all the other components evaluated (Table 3), that is, the greater plant height and the greater number of pods, result in greater number of seeds. In a study by Bertoldo et al. (2009), on the correlation of bean production characters, similar results were observed in terms of the number of seeds per plant in relation to the other characters studied.

The only component that showed a negative correlation was the number of pods with the first pod insertion height, which is a low magnitude correlation. There was also a correlation of average magnitude between the number of pods and the plant height, representing that the greater the plant height, the greater the number of pods.

Table 3. Pearson's linear correlation for the characters studied first pod insertion height (PI), plant height (PH), number of pods per plant (NP), number of seeds per plant (NS), seed mass per plant (SM) of beans segregating populations and families, conducted in the county of Tenente Portela, Brazil, 2019.

	PI	PH	NP	NS	SM
PI	.	0.23*	-0.10*	0.16*	0.08 ^{NS}
PH		.	0.47*	0.45*	0.02 ^{NS}
NP			.	0.79*	0.03 ^{NS}
NS				.	0.19*
SM					.

^{NS} For non-significant values; * For significant values at 5% by the t test; n = 670.

Neural networks

For the study carried out, the Kohonen mapping method was used, using 134 inputs (neurons) with thousands of interactions, this matrix was subjected to the procedures that defined a neural network with 15 centroids, establishing associative patterns among the tested segregating generations, in a way that 17 synaptic connections were needed to connect the centroids.

Selection based on the centroid results in the greatest similarity between individuals so that

through the technique it was possible to distinguish 15 association groups (Figure 4, Table 4). The centroid with the largest number of individuals was XIV with about 10.44% of individuals, followed by Centroides III, XII with 9.7% each, centroid XIII with 8.95%, centroid V with 7.46%. Centroids VII, IX, X, and XV obtained 5.97% of individuals each, centroid II and IV 5.22% each, centroid VI and VIII 4.47% each. Centroids XI and I had the lowest number of individuals, corresponding to about 3.73% of the total number. Based on the 15 centroids selected, it is possible to make selections for certain agronomic aspects, with the possibility of identifying the centroid of interest and consequently the corresponding group of genotypes or neurons. Corroborating with Theodoro et al. (2015), that Artificial Neural Networks can be considered a very effective alternative to measure phenomena, of genotypes in breeding programs. In the same sense, Nascimento et al. (2013) argue that neural networks can retain the most complex characteristics of existing data sets, and therefore do not require detailed information, which can be attributed by self-learning.

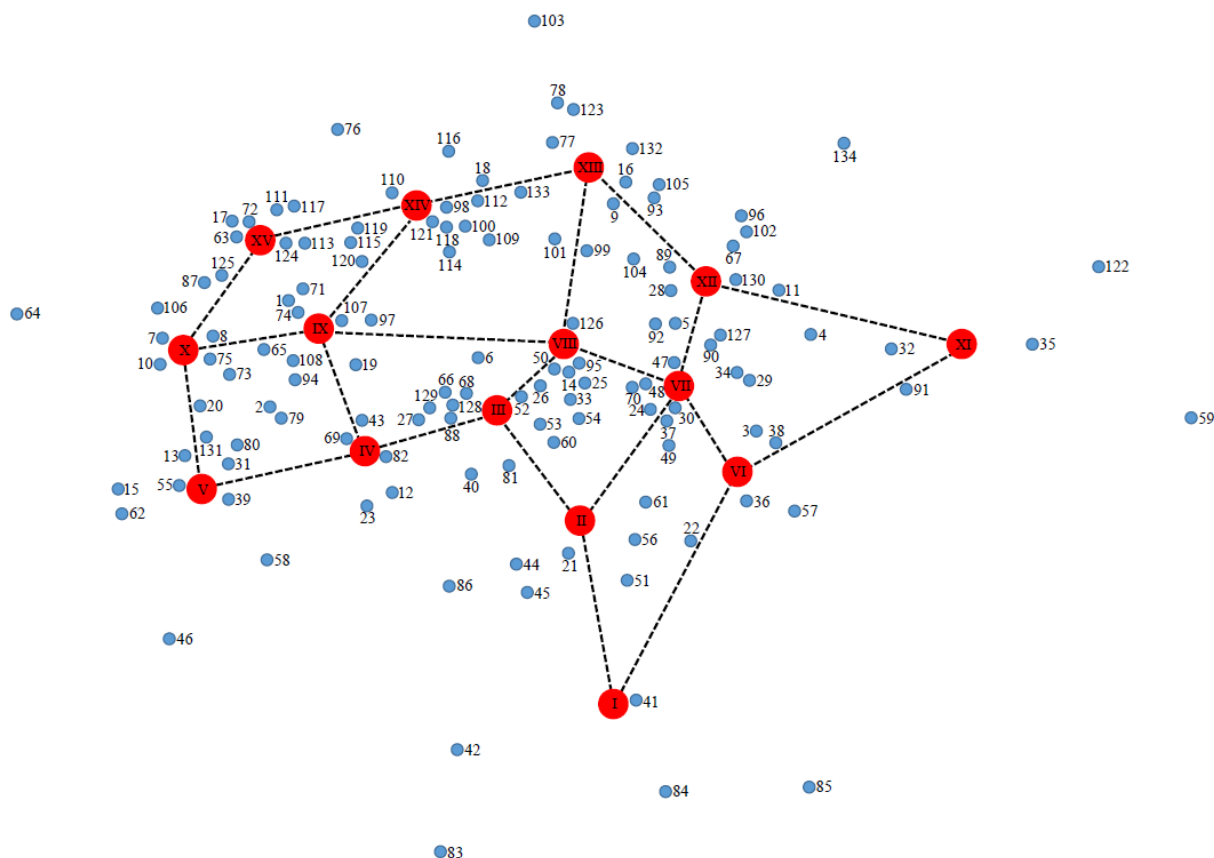


Figure 4. Artificial Neural Networks (ANNs) obtained by the Kohonen Map defining the centroids (red) and the neurons of interest (blue) and the synaptic connections (blue lines).

Table 4. Kohonen's Mapping Method of selected individuals in certain centroids.

Kohonen'smap	Individuals	Total Individuals
CentroidI	41 42 83 84 85	5
CentroidII	21 44 45 51 56 61 86	7
Centroid III	6 26 40 52 53 54 60 66 68 81 88 128 129	13
CentroidIV	12 23 27 43 69 79 82	7
CentroidV	13 15 31 39 46 55 58 62 80 131	10
CentroidVI	3 22 36 38 49 57	6
CentroidVII	24 29 30 34 37 47 48 70 90	9
CentroidVIII	14 25 33 50 95 126	6
CentroidIX	1 19 65 71 74 94 97 107 108	9
CentroidX	2 7 8 10 20 64 73 75 106	9
CentroidXI	32 35 59 91 122	5
CentroidXII	4 5 11 28 67 89 92 96 102 104 127 130 134	13
CentroidXIII	9 16 77 78 93 99 101 103 105 123 132 133	12
CentroidXIV	18 76 98 100 109 110 112 114 115 116 118 119 120 121	14
CentroidXV	17 63 72 87 111 113 117 124 125	9

Conclusion

After the characterization of the 134 genotypes, it was possible to verify that through Singh's relative contribution (1981), the character of number of seeds per plant was the most decisive for the genetic variability of this study.

The use of biometric methods allows it to be possible to evidence patterns in relation to the genotype clusters, through the use of standardized

average Euclidean distance patterns, Tocher's optimization method, UPGMA clustering method, and Artificial Neural Networks with unsupervised learning.

With the results obtained, it was possible to verify the existence of genetic variability among the studied populations, allowing from the cluster analysis to delimit crossings between accessions with genetic potential for the formation of superior genotypes.

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