

Phaseolus lunatus L.: pulse seeds phenotype image analysis*

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Abstract

Phaseolus lunatus L. is a species with great diversity, which can be explored in breeding programs when adequately characterized. In this work, we evaluated the phenotypic variations of *P. lunatus* seeds by image analysis using seeds from 13 genotypes from three states: Sergipe (G1, G2, G3, G4, G5, G6, G7, and G8), Bahia (G9 and G10) and Alagoas (G11, G12, and G13). For the analyses, the weight of 100 seeds was estimated, and, in the Groundeye (TBit®), the morphometry by area (mm²), maximum diameter (mm) and minimum diameter (mm), and the descriptors dominant color and RGB color system. The morphometric variables were submitted to variance analysis by the F test, and the means clustered by the Skott-Knott test at 5%. The data were submitted to Pearson correlation analysis (t-Student at 5%), grouped according to dissimilarity by the UPGMA, and represented in the dendrogram. The evaluated characters were submitted to principal component analysis (PCA). Concerning the dominant color, there was a predominance of orange in nine genotypes. As for the RGB color system, G1 presented the highest values and G11 the lowest. G7 was superior for morphometric variables, except for the weight of 100 seeds. There is a positive and significant association for morphometry. There are two homogeneous and distinct groups in the dendrogram, and the first two principal components were responsible for 86.80% of the genotypes' variation. Therefore, applying high-resolution images for phenotypic characteristics of creole bean seeds is a promising nondestructive tool aiming for selection.

Introduction

Fava (*Phaseolus lunatus*) is an agricultural crop of great economic interest. This legume is considered the second most important species of the genus *Phaseolus*, cultivated in various places in the world due to its tolerance to unfavorable edaphoclimatic conditions (Noh et al. 2015). A phylogenetic analysis performed with *P. lunatus* noted that the species is more closely related to the species of beans *P. vulgaris*, *Vigna unguiculata*, and *Vigna radiata* (Tian et al. 2021).

The main form of conservation is on-farm by small farmers who store their seeds, preserving genetic diversity. Conservation on-farm is an alternative to diversifying agriculture but requires government and private support to stimulate and improve agricultural interaction among smallholder farmers (Soares et al. 2021). In Brazil, its cultivation occurs in the Northeast region, mainly carried out by family farming, due to its adaptation to water scarcity, high temperature, and low fertility soils (Soares et al. 2021; Lee et al. 2015).

Fava, commonly sold at free fairs, is part of the food base of many communities. However, there is considerable heterogeneity in the characteristics of the grains produced in these communities, which receive different nomenclatures depending on the region (Félix et al. 2014). The species presents high phenotypic variability regarding the habit of growth (determined or indeterminate), flower color (white, yellowish-white, pink, purple, and violet and with intermediate tones), and color of the seed integument (white, gray, yellow, pink, brown, black, among others). In addition, there are variations in the shape (rhomboid, round or reniform), size, and weight of the seeds, among other morphometric characteristics (Lopes et al. 2015). However, these characteristics still need to be explored by farmers since there are few studies on selecting characters of interest in participatory breeding programs. Because of this, the materials available for cultivating small farmers are a varietal mixture. These have low productivity and uneven characteristics such as tegument color and size, which is unsuitable for grain commercialization (Silva et al. 2015).

The genetic diversity of Creole varieties accessed at the Phaseolus Germplasm Bank of the Federal University of Piauí (PGB-UFPI, Brazil) identified 26 qualitative and 11 quantitative characters, following the descriptors of Biodiversity International. In addition, SSR (Simple Sequence Repeats) molecular markers were used to determine genetic polymorphism and differentiation. Considerable geno-phenotypic diversity between the accesses for the number of pods per plant, the number of seeds per pod, and the weight of 100 seeds were identified (Pires et al. 2021).

Thus, morphometric characterization studies are an essential strategy to select traces of economic interest according to the consumer market demand. In addition, using image analysis equipment results in greater accuracy of the evaluated characters, consequently, more significant genetic progress in the selection process. Therefore, the objective of this work was to evaluate the morphometry and staining of the tegument of *P. lunatus* creole bean seeds by image analysis.

Material And Methods

The seeds used in the experiments are from three states: Sergipe (G1, G2, G3, G4, G5, G6, G7, and G8), Bahia (G9 and G10), and Alagoas (G11, G12, and G13). The genotypes of the states of Sergipe and Bahia were acquired from family farmers in the municipalities of Frei Paulo-SE and Pedro Alexandre-BA and stored in bottles. The Federal University of Alagoas (UFAL) - Rio Largo-AL donated seeds kept in a cold chamber at 8°C ± 2. Initially, 100 seeds of each genotype were weighed on a precision scale to access the weight of 100 seeds (WHS), according to the recommendation of the International Plant Genetic Resources Institute - IPGRI (2001).

Morphometric analysis used four replicates of 25 seeds per genotype, performed with the aid of the Groundeye (TBit®) equipment, calibrated before capturing images. From the captured images, the variables of area (mm²), maximum diameter (mm), and minimum diameter (mm) in software was used for the most detailed classification of seeds by color, using the dominant color and RGB color system descriptors (red, green, and blue).

The morphometric data were evaluated by normality and homoscedasticity of variances. By the F test, we evaluated the treatment significance and the means clustered by the Scott-Knott test (5%). Pearson's correlation was performed using the t-test at 5% probability to verify morphometric characters' degree of dependence. The dissimilarities by Unweighted Pair Group Method using arithmetic averages (UPGMA) assessed all genotypes. The distinctions were clustering by the dendrogram and a column chart plotted using excel software® to assist the dominant color and the RGB system.

Principal component analysis (PCA) was performed using the descriptors: area (mm²), maximum diameter (mm) and minimum (mm), and RGB color (Manly 2008), with z-score standardization (Regazzi 2000). Statistical analyses were performed in the GENES® software (Cruz 2013) and R® version 4.1.3.

Results And Discussion

The high-resolution images performed in the Groundeye equipment (TBit®) are in Fig. 1.

According to the Groundeye (TBit®) analysis, there was a predominance of orange (G2, G3, G5, G6, G7, G8, G9, G12, and G13), yellow (G1 and G10), black (G13) and red (G4) (Fig. 2). The camera allowed us to capture the predominant color of the seeds of each genotype. In turn, in most genotypes, it is distinct from the color observed by the human eye. The difference between the human eye and Groundeye is that the equipment uses complex algorithms to differentiate colors, and the human eye cannot make this distinction.

According to the data in Fig. 2, nine categories predominated orange, with G5 presenting the highest value (95.4%) and G12 the lowest (53.36%). In G1, the highest dominance of yellow (95.98), and in G10 was the lowest (87.96%), while in G4, the dominant color was red (49.77%), and in G11, it was black (83.44%).

The capture of the images allowed us to visually verify that the genotypes present high phenotypic variation in the color of the tegument. In G6, small black spots were observed in the seeds, suggesting variable expressiveness and incomplete penetrance. That occurrence can explain why this genotype has the highest number of outliers (Fig. 3) since the scores distributed in each seed have shapes and sizes. In addition to G6, G1 and G10 also have more outliers (Fig. 3). The outlier's occurrence may be related to dirt and physical damage in the tegument since they are white (visually). This evidence leads to generating more discrepant values if these factors are present.

The subjectivity eliminated allows, through values, to quantify phenomena, such as gene expression, from qualitative characteristics, such as color. Therefore, image analysis in seed phenotyping made it possible to transform qualitative characteristics into quantitative ones. The knowledge of this information is a valuable tool for the characterization, registration, and identification of variations (Lopes et al. 2010).

This characteristic is directly related to the commercialization of fava seeds since the preference is for white seeds (yellow dominance in Groundeye). The color is an essential characteristic for the distinction of varieties used by farmers during empirical selection (Soares et al. 2021).

The seeds have a great diversity of colors, such as: white, brown, gray, yellow, pink, red, purple, black, stained, and mottled (Moraes et al. 2017). This qualitative characteristic is of great economic importance because it is linked to the acceptance of grains by consumers since they associate the lighter coloration with a material with a less bitter taste, besides being related to recent harvest and, consequently, easy to cook (Lopes et al. 2010, Lopes et al. 2015).

Thus, it is necessary to consider different approaches that contribute to the conservation of creole varieties of broad beans (Soares et al. 2021) and actions that allow a better orientation of farmers in obtaining marketable seeds.

There is a precise color seed classification on the RGB color system using the software on the red (R), green (G), and blue components (Fig. 4).

Components R, G, and B are the amounts of red, green, and blue light, with measured values ranging from 0 to 255 (Tsuchiyama and Matsushima 2017). According to Fig. 2, G1 presented the highest values of RGB and G11 the lowest, 200.82, 147.193, 193.26, 45.77, 30.25, and 40.86, respectively.

The minimum value (0) indicates dark, while the maximum demonstrates high intensity (clarity). When all colors are at the minimum value, the resulting color is black, but if all are at the maximum value, the color is white. If all values are similar or very close, the tone is gray. However, if values tend to distance themselves, the result will be an increasingly "alive" and less "soft" tone (Fernandez-Rodriguez 2017).

Based on this, G1 and G10, with the dominant yellow color, approached the white color, while G14 and G11, with the dominant color red and black, tended to the black tone. G9, G12, and G13 presented similar RGB values, with the tegument color being gray. G3 and G7, visually presenting the gray color, had more significant variations in RGB values due to stains in the tegument.

For the RGB color system, G1, G3, G5, G7, G8, G9, G10, G12, and G13 can be classified as light tegument seeds; while genotypes G2, G4, G6, and G11 are close to dark colors (Sunoj et al. 2018).

Thus, the light tegument genotypes were predominant. It may be related to the fact that this characteristic is exploited by farmers when selecting seeds since the consumer market demands lighter coloration materials, thus eliminating the varieties with dark integuments (Soares et al. 2021).

For the morphometric variables AR and WHS of the seeds of the 13 genotypes, significant differences ($p < 0.01$) were verified (Table 1).

Table 1
Mean values and coefficient of variation of morphometric variables of *Phaseolus lunatus* L. AR - area seeds; D.Max - maximum diameter; D.Min - minimum diameter; WHS - the weight of 100 seeds.

Genotype	AR (mm ²)	D.Max.(mm)	D.Min.(mm)	WHS (g)
G1	86.88c	12.03a	8.36a	45.33a
G2	65.71g	10.43a	7.65a	29.77f
G3	71.01f	11.37a	7.68a	34.14d
G4	79.71e	11.56a	8.51a	40.74b
G5	71.06f	10.88a	7.95a	31.63e
G6	72.38f	11.09a	7.94a	31.96e
G7	93.65a	12.69a	9.23a	40.67b
G8	89.09b	12.08a	8.75a	41.59b
G9	81.89d	12.31a	8.26a	40.74b
G10	71.46f	11.20a	7.79a	29.55f
G11	66.33g	10.42a	7.78a	38.73c
G12	80.22e	11.45a	8.75a	33.95d
G13	63.87h	10.58a	7.46a	33.60d
Média	76.41	11.39	8.16	36.34
CV (%)	2.67	1.70	2.22	1.40

*Followed averages of the same letter do not differ statistically from each other in the column by the Scott-Knott test at 5% probability.

According to the data (Table 3), the area presented mean values between 93.65 mm² (G7) to 63.87 mm² (G13), and genotype G7 was statistically superior to the others. Regarding the maximum diameter, the mean values ranged from 12.69 mm (G7) to 10.42 mm (G11), while in the minor diameter, there were no significant differences in both variables.

Regarding the WHS, the values varied between 45.33 (G1) and 29.55g (G10), and Genotype G1 was statistically superior to the others. In addition, the morphoagronomic characteristics showed a low coefficient of variation (CV (%)). Using images to evaluate these characters allows for reducing measurement errors throughout the evaluation, so residual systematic errors are minor.

This species' differentiation in seed weight is vital (Félix et al. 2014). This variable is pointed out in studies developed with the species as one of the quantitative characteristics that most contributes to the analysis of genetic divergence, ranging from 70% (Meza et al. 2012) to 97.32% (Lopes et al. 2010).

Genotype G13 prevailed among the lowest mean values in most parameters. Therefore, it is composed mainly of smaller seeds. In G7, the highest values are present, thus having a predominance of seeds with larger dimensions.

In a genetic study with 300 accesses of *P. lunatus* from different origins in Honduras, provided by farmers, there is great diversity among genotypes and seed dimensions (Meza et al. 2012). The variation in other studies, with high variability in the size, color, and shape of seeds

(Guimarães et al. 2007; Lee et al. 2010).

In the simple correlation analysis (Pearson) is a positive and significant association between all morphometric variables (Fig. 3).

The correlation between agronomic characters is of great interest to breeding programs because it allows evaluation of the degree of interference of one attribute over another, enabling indirect selection. In the case of simple correlation, the evaluation is the degree of relationship between two numerical variables, one dependent (Y_i) and one independent (X_i) (Petter et al. 2014).

Thus, it is possible to infer that there is potential for indirect selection for the weight character of 100 seeds (WHS) through the evaluation of AR, D.Max, D.Min, AR, and WHS, regardless of the genotype of *P. lunatus*.

The results obtained (Fig. 3) present a linear correlation of strong ($0.6 < r \leq 0.9$) to very strong ($0.9 < r < 1$) degree (Callegari-Jacques 2009). The event may be related to pleiotropy or gene binding, a property in which a gene affects two or more characteristics. In this case, the effect is positive since some genes influence the increase of both characteristics (Falconer and Mackay 1996).

From the point of view of genetic breeding, the characteristics AR, D.Max, D.Min, AR, and WHS have a positive correlation, so the emphasis on selection should be performed only in one for the improvement of the two characters. Thus, the number of characteristics to be evaluated can be reduced by optimizing the time and breeder work (Nogueira et al. 2012).

According to the Mojena method's (1977) with $k = 1.25$, two accesses groups were comprised in the dissimilarity dendrogram using morphometric characteristics (Fig. 4).

Group A consists of seven genotypes (G2, G3, G5, G6, G10, G11 and G13) and B by six (G1, G4, G7, G8, G9 and G12). The groups are responsible for 79.97% of genetic divergence, with 25.6%.

The most important group for the dissimilarity of the accesses is B since it is the most divergent, being the one with the genotypes with higher means of AR, D.Max, D.Min, and WHS. The divergences observed among the genotypes of *P. lunatus* are significant, as it is a strong indicator of genetic diversity among the varieties. Using these materials in plant breeding programs to obtain materials with characteristics required by the interested consumer market is essential.

Dissimilarity measures have high relevance in genetic diversity studies, especially when sourcing to identify parents for hybridization programs. This technique allows for obtaining homogeneous groups through a scheme that allows grouping the genotypes. The objective is to identify high homogeneity within groups and heterogeneity among them (Cruz et al. 2011).

In the principal component analysis (PCA), the correlation coefficients with the two main components (CP), auto values, total variance, accumulated auto values, and the accumulated total, as well as the associated autovectors, are presented for each principal component (Table 4). In the main component, 1 (CP1), the variable that most influenced its formation was AR (0.92), and in the second, the R (0.72).

Table 2

Values of the correlation coefficients with the two main components (CPs), auto value, total variance, accumulated auto values, and total accumulated in percentage (%) by the components, of the thirteen genotypes of *Phaseolus lunatus* L., from the morphological and colorful characters.

Variable	CP 1	CP 2
AR	0.92	-0.36
D.Max	0.95	-0.20
D.Min	0.74	-0.58
WHS	0.67	-0.50
R	0.65	0.72
G	0.69	0.60
B	0.67	0.60
Autovalues	4.09	1.99
Total variance (%)	58.41	28.39
Accumulated autovalues	4.09	6.08
Total accumulated (%)	58.41	86.80

The contribution of a principal component is analyzed through the proportion of total variance explained by the component (Regazzi 2000). Therefore, the first two CP were responsible for 86.80% of the total variation on the variables (morphological and staining) of the different seed groups of *P. lunatus*. Therefore, the first two PCs effectively summarize the total sample variance and can be used to analyze datasets.

The result obtained is by the value proposed by Rencher (2002), which defines that the first and second CP should explain at least 70% of the total variance. In this study, PC1 accounted for 58.41%, and PC2 accounted for 28.39% of data variations. The variables related to morphological characteristics have a high correlation with PC1, while those of the RGB color system highly correlate with PC2 (Callegari-Jacques 2009).

In a study with 47 cowpea genotypes (*Vigna unguiculata* L.) of various origins, the divergence combining different characteristics such as weight of 100 seeds, grain shape, and grain color, among others, was used three main components to explain 80.32% of the total variance. The application of this tool allowed the identification of 13 genotypes with superior characteristics in production, precocity, and grain quality (Bertini et al. 2010).

Figure 6 shows that the positive correlations place genotypes G1, G3, G7, G8, G9 and G12 to the right of CP1, while the negative correlation places genotypes G2, G4, G5, G6, G10, G11 and G13 to the left of CP1. Regarding CP2, genotypes with positive correlation are located at the top (G1, G2, G3, G5, G9, G10, and G13), while genotypes with negative correlation are present at the bottom (G4, G6, G7, G8, G9, G11, and G12).

According to the biplot graphics, the variables associated with morphological and RGB characteristics face the right of CP1. Genotypes G1, G3, G7, G8, G9, and G12 (CP1) have the potential for higher morphometric values, more intense tones, and light colors. In the case of CP2, genotypes G1, G2, G3, G5, G9, G10, and G13 present higher values for RGB, more intense shades.

According to the biplot graphics, the variables associated with morphological and RGB characteristics face the right of CP1. Genotypes G1, G3, G7, G8, G9, and G12 (CP1) have the potential for higher morphometric values, more intense tones, and light colors. In the case of CP2, genotypes G1, G2, G3, G5, G9, G10, and G13 present higher values for RGB, more intense shades.

It is essential to look at several factors for a breeding program to succeed. In the case of *P. lunatus*, the consumer market is highly relevant because for a genotype to consider of interest, it must contain bulky grains with lighter coloration. In the Northeast region, consumer preference is for brown and white materials (Soares et al. 2021).

Thus, according to the results of the principal component analysis, genotypes G1, G3, and G9 have morphological and visual characteristics, such as large seeds and clear tegument, which meet the requirements demanded by fava bean consumers. The genotypes can be used in plant breeding programs.

Conclusion

Using high-resolution images to evaluate morphological characters and color of the creole bean seed tegument proved to be a promising tool for analyzing phenotypic characters. Multivariate analysis of the data obtained with image processing makes it possible to infer that genotypes G1, G3, and G9 have morphological and visual characteristics that meet the interests for use and selection in plant breeding programs.

Declarations

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Ethics declarations

Conflict of interest

All the authors of this manuscript declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

SJD conducted the research and wrote the text, MFOT contributed to obtaining the research data. RSM and PFV guided the research, revised the text and contributed to the writing of the manuscript in the writing, translation and revision of the text. All authors contributed to the article and approved the submitted version.

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Figures



Figure 1

Image of *Phaseolus lunatus* L. genotypes obtained in GroundEye (TBit®). G= genotype.

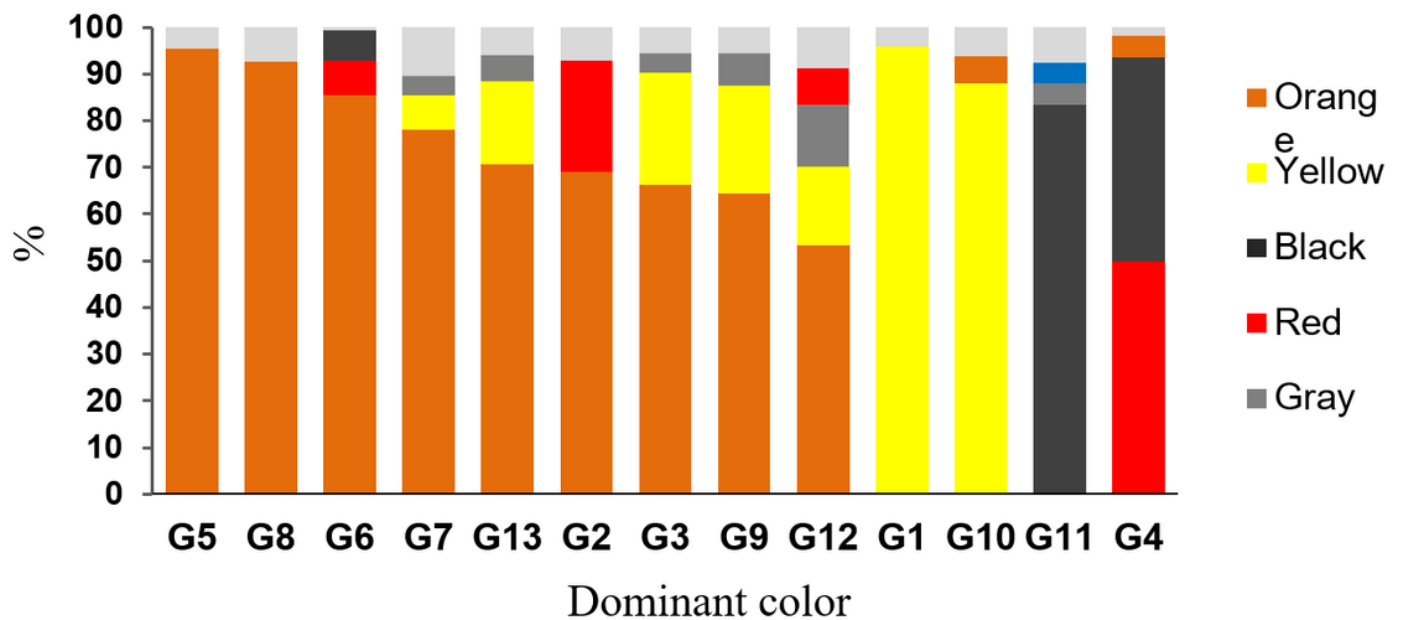


Figure 2

Dominant color of the tegument (%) of *Phaseolus lunatus* L. in GroundEye (TBit®). *Colors $\leq 4\%$ were included in the other category.

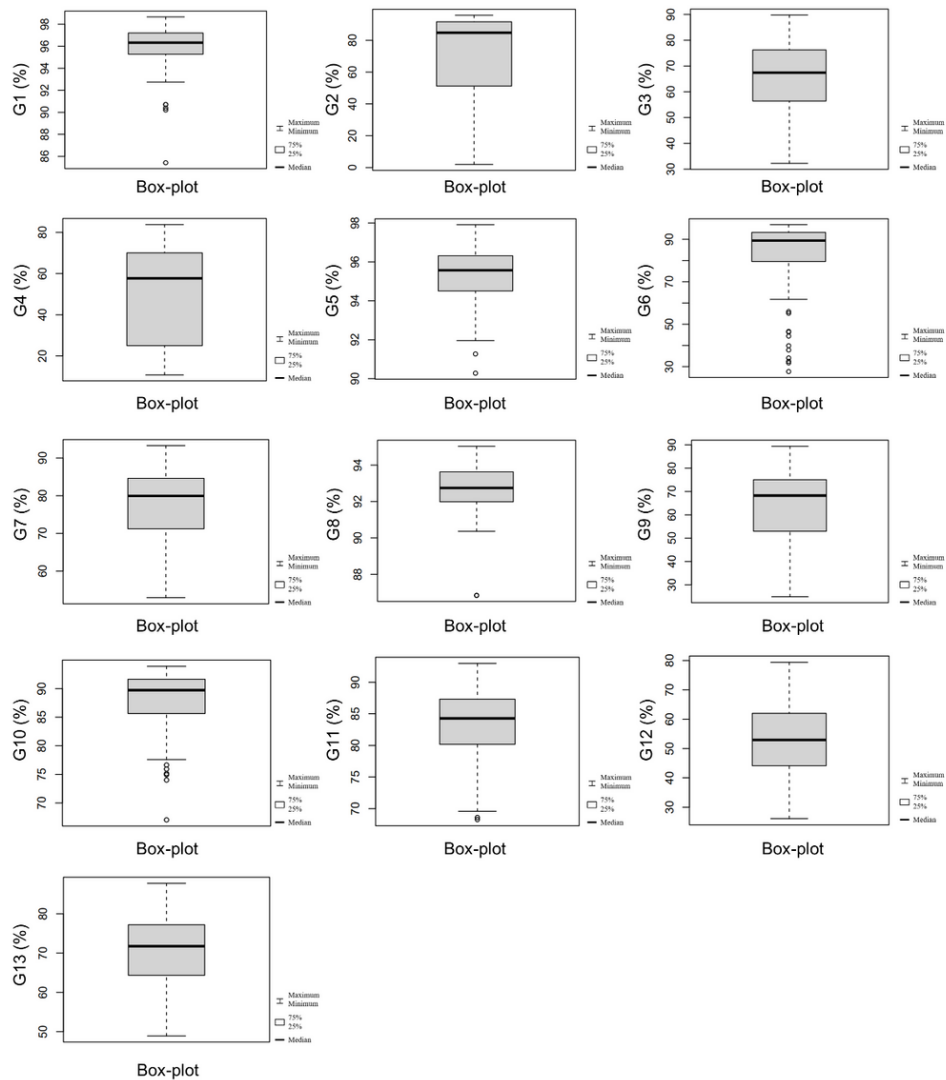


Figure 3

Dominant color of the tegument (%) of the genotypes of *Phaseolus lunatus* L. obtained through GroundEye (TBit ®).

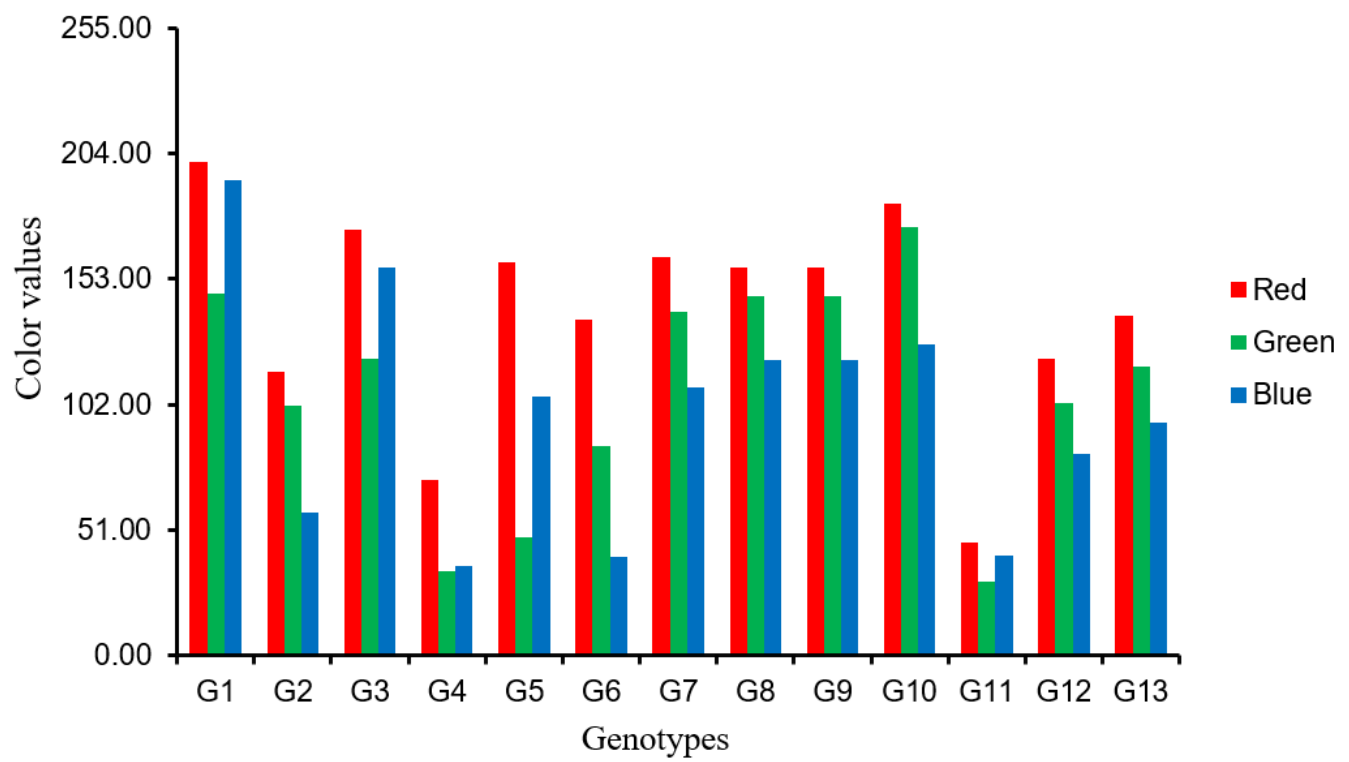


Figure 4

RGB, R - red, G - green, and B - a blue color system of the 13 genotypes of *Phaseolus lunatus* L., obtained through GroundEye (TBit ©).

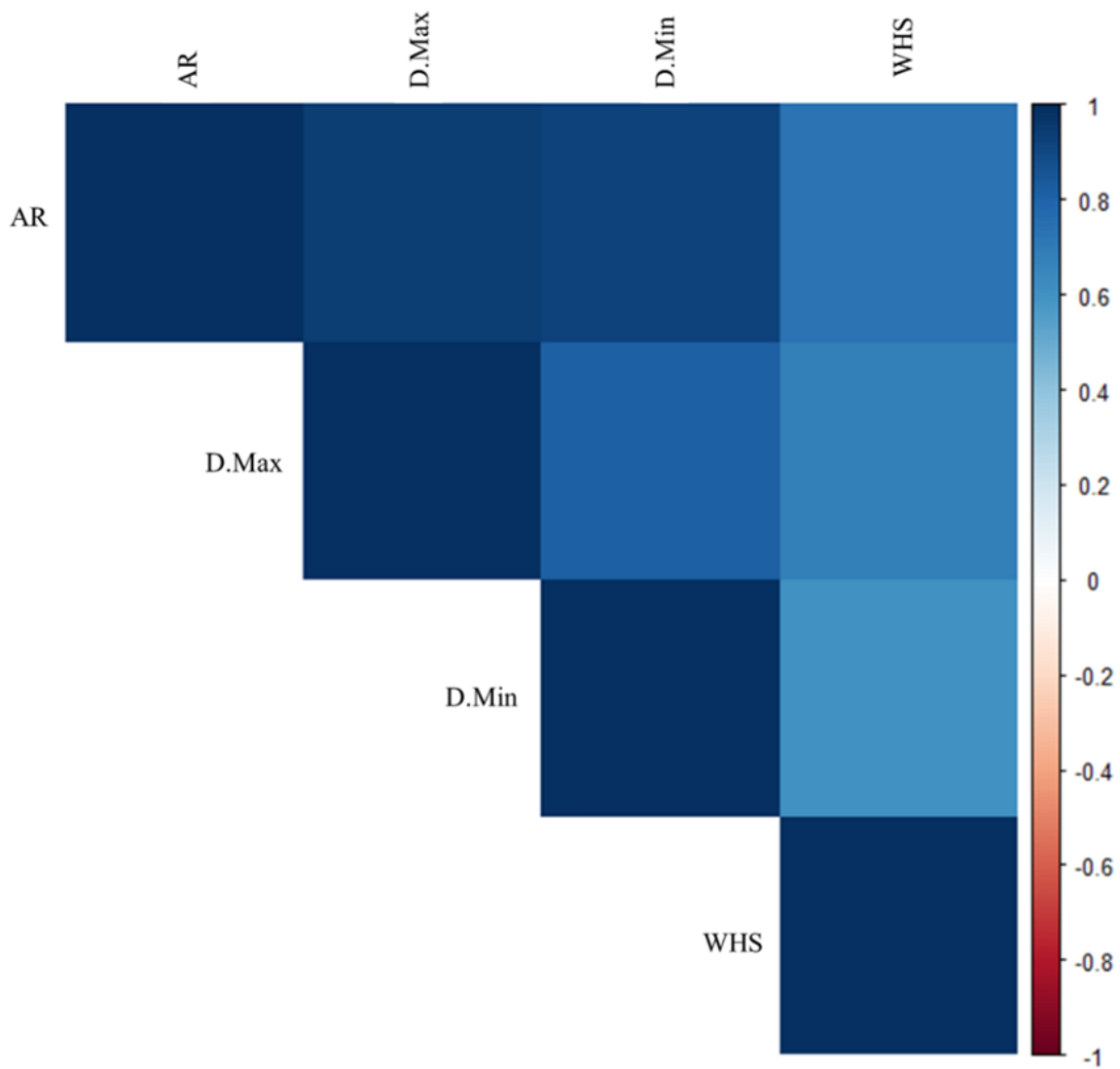


Figure 5

Pearson correlation matrix (5%) between morphological variables of *Phaseolus lunatus* L seeds.

Dissimilarity and cut-off point among genotypes

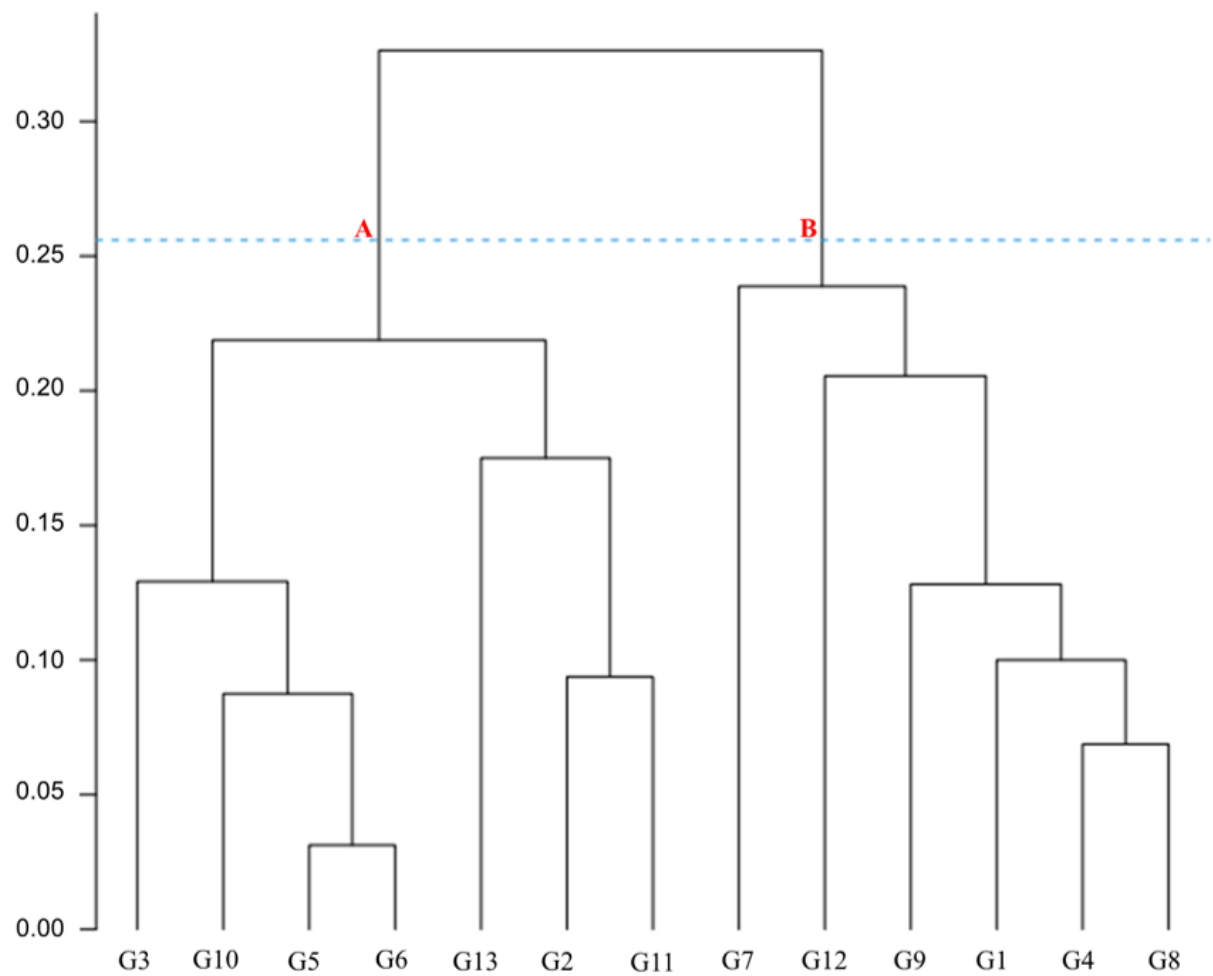


Figure 6

Dissimilarity dendrogram among genotypes (G) of *Phaseolus lunatus* L., based on the Euclidean distance and clustered by UPGMA.

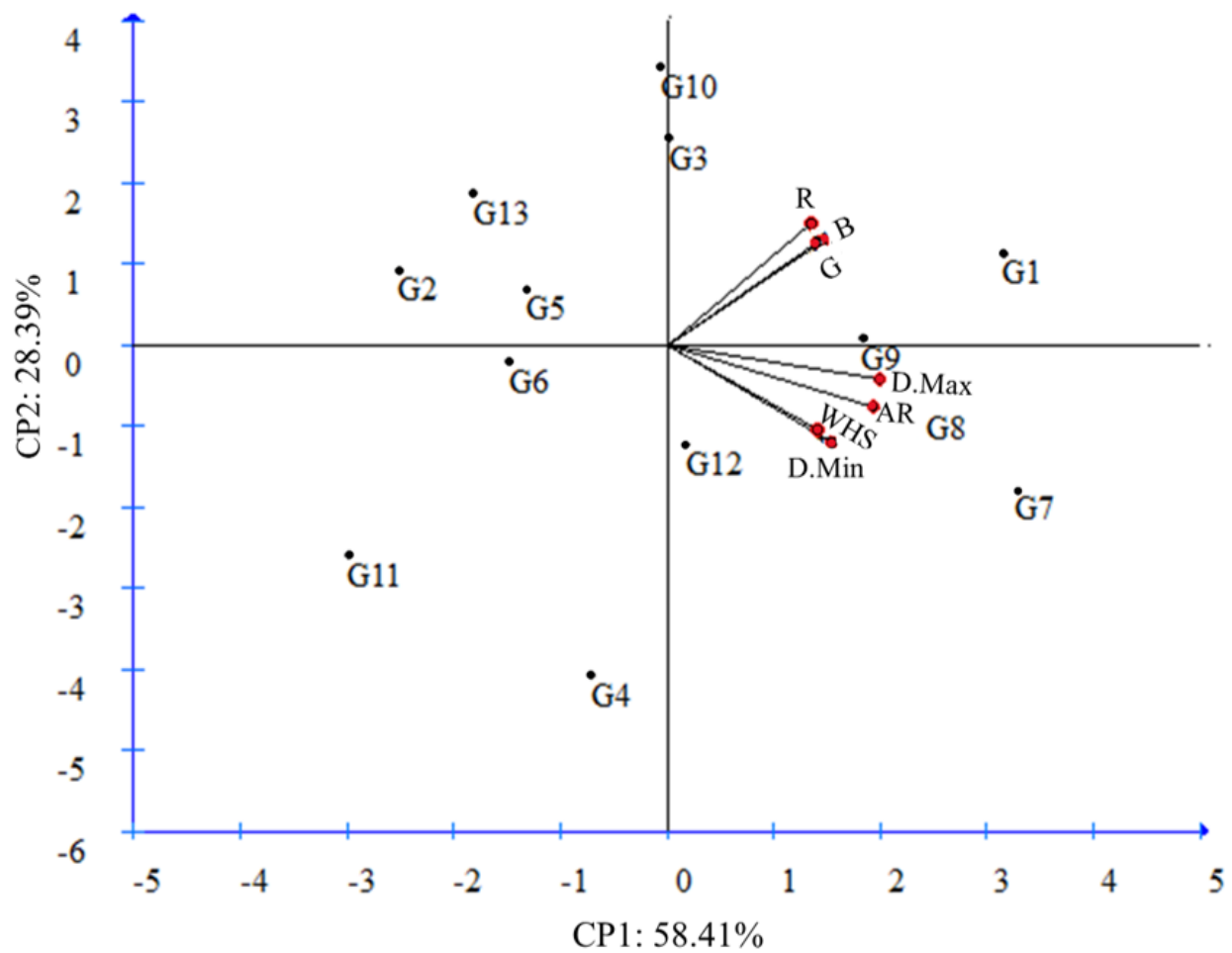


Figure 7

Biplot with the projection of the variables of the first two principal components of the 13 genotypes of *Phaseolus lunatus* L., obtained by the Method of principal component analysis, from the morphological characters and color.