

Similarity networks in genotypes of *Vigna unguiculata* (L.) Walp for green-grain production

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Abstract

Cowpea is one of the most significant food and nutrient sources worldwide, with importance in three primary market sectors: dry grains, seeds, and the expanding green-grain sector. This study aimed to identify phenotypic patterns for selection in *Vigna unguiculata* (L.) Walp genotypes for green-grain production using similarity networks and identify its morpho-agronomic variables with greater discrimination ability. The rainfed experiment was conducted in the experimental area of the horticultural sector at the Plant Science Department of the Agricultural Sciences Center of the Federal University of Ceará, Brazil, with 42 treatments. Three seeds were sown per hole, and the plants were thinned to two plants per hole, 15 days after sowing. Characterization was performed based on quantitative and qualitative variables, and the data were subjected to multivariate analysis of variance based on an augmented block design. The conjugate distance matrix for the variables was graphically represented using similarity networks to identify phenotypic patterns. The results indicated that genotypes CE-164, 207, 999, 994, 1002, and 1007 should not be used in breeding programs for green-pod production since they show genetic similarity within commercial cultivars. The variables of days to fruiting, green-pod length, green-pod width, green-pod thickness, and green-grain thickness contribute to genetic divergence and have high heritability values. Crosses between cowpea genotypes CE-165, 244, 22, 96, and 98 can yield gains in green-grain production in advanced generations.

Keywords: Cowpea, multivariate analysis, plant breeding, selection, variability.

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is an annual, herbaceous plant grown mainly in dry tropical regions of Latin America, Africa, and South Asia (Boukar et al., 2019), where it is one of the most important food and nutrient sources (Correa et al., 2015; Silva et al., 2018).

From the social and economic perspectives, this crop is one of the main food options cultivated in Brazil, contributing to income generation for farming families, especially in the North and Northeast regions (Sousa et al., 2017). In addition, cowpea cultivation has expanded in the same areas used for commercial crops (Silva et al., 2016) by medium and large entrepreneurs, predominantly in the off-season (Oliveira et al., 2017). However, despite the high cowpea production in Brazil, the grain supply of this crop is still insufficient since its mean yield is considerably low (Torres et al., 2015).

Cowpea grains are rich in proteins (20 - 25%), carbohydrates, folic acid, and various minerals, constituting the most important part of the harvest, although young leaves and immature fruits can also be consumed (Boukar et al., 2016). In addition, the various uses of this species comprise of three major market sectors: green beans (pods and grains), dry grains, and seeds (Sousa et al., 2015), with the green-bean sector showing increasing importance (Almeida et al., 2019).

In this scenario, due to its significant importance in Brazil, especially in the Northeast region, studies are needed to assess cowpea genetic divergence, select genotypes for breeding programs (Santos et al., 2014), and classify this material for use in different agronomic areas (Ferreira et al., 2020), e.g., the green-grain market.

Creating new varieties with specific traits requires knowledge about the materials at hand in germplasm banks in order to select genotypes with wide genetic diversity, thereby meeting the objectives of selection (Xiong et al., 2016). The screening of a wide range of genotypes in breeding programs includes the measurement of several significantly correlated variables, thus favoring the use of multivariate analysis (Grigolo et al., 2018; Souza and Nascimento, 2020).

Similarity networks are among the multivariate methods with high potential for identifying genotypic patterns, consisting of a two-dimensional representation of an n -dimensional distance matrix. The network consists of connections in which each edge includes a weight that indicates the strength of the correlation. The stronger the correlation, the thicker the connecting line (Silva et al., 2016) and, consequently, the more similar the evaluated genotypes. Although it may provide a graphic representation of superior individuals, this technique is less used in genetic

divergence studies with plants than clustering or multidimensional scaling.

From this perspective, this study aimed to identify phenotypic patterns for selection in cowpea genotypes (*Vigna unguiculata* (L.) Walp) meant for green-grain production using similarity networks and identify the morpho-agronomic variables with greater discrimination capacity.

Results and Discussion

Relative contribution of the variables

Five of the fifteen quantitative variables contributed 71.4% of the genetic divergence among cowpea genotypes (*V. unguiculata* (L.) Walp.) (Table 1). Identifying the most important traits for genetic dissimilarity estimation is a viable alternative since it is unfeasible to evaluate many traits with a large number of genotypes (Aquino et al., 2017), as seen in the method proposed by Singh. Therefore, variables that can effectively discriminate between genotypes can be prioritized to recommend cowpea genotypes meant for increased green-grain production.

Similar to the present study, Sousa et al. (2017) reported that some cowpea traits such as yield, days to pod maturity, and the number of grains per pod contributed the most to the dissimilarity between genotypes, revealing to be useful and effective traits to select parents in a breeding program for the species. In this scenario, according to Lima et al. (2018), one of the first steps when breeding plants is to identify genotypes with superior or desirable variables to develop new cultivars.

The variables that least contributed to this dissimilarity were the number of loculi per pod, the number of grains per pod, and the width of green grains (Table 1), which can be disregarded in future studies. When the variables under analysis contribute slightly to the genetic diversity of the population, they are not relevant to this type of study, although their evaluation is important so that the productive potential of the genotype can be assessed (Grigolo et al., 2018).

Heritability

The heritability values were obtained in boxplots by estimating the genetic basis obtained from the mean of the genotypes for each quantitative variable (Fig. 1). The width of the box (vertical) is proportional to the broad-sense heritability of each trait (Silva et al., 2016).

Green-pod width (0.86), green-pod thickness (0.86), days to fruiting (0.83), green-grain thickness (0.82), green-grain length (0.78), and green-pod length (0.77) showed high heritability values (Fig. 1), implying little environmental influence and the predominant control by genetic factors. Therefore, these traits can be used in breeding programs to select superior individuals (Kampa et al., 2020) and transmit the features to the next generation (Chaves Neto et al., 2020). On the other hand, selection will be slower and ineffective for traits with low heritability due to environmental differences between individuals (Siqueira et al., 2013), favoring their use for selection in advanced generations (Leite et al., 2016).

The variables of plant height, days to flowering, stem diameter, number of nodes on the main branch, green-pod weight, number of loculi per pod, number of grains per pod, and green-grain thickness showed the lowest heritability values (Fig. 1), with green-grain thickness (0.01) showing the

lowest value among these traits, close to zero. These traits did not promote any variability between genotypes, either due to some environmental influence on phenotypic manifestation or because the evaluated genotypes showed little variability for them. Heritability is null when values are close to or equal to zero, as seen in green-grain thickness (0.01), probably due to the high environmental influence (Silva et al., 2010). Several factors influence heritability estimates (Borém and Miranda, 2013), and some ways to increase heritability values would be to consider the sample size, the type of environment or plot, experimental design, and the number of genotypes in the population.

Similarity network between genotypes

Fig. 2 shows the genetic similarity between cowpea genotypes and commercial cultivars (highlighted in the figure) based on the quantitative and qualitative variables under evaluation. The similarity network analysis detects more-similar groups through closer interactions (Kumar and Deo, 2012), where each line represents an association between two or more traits (Epskamp et al., 2012) and/or genotypes.

Dispersion was found between most genotypes, highlighting their divergence and indicating that these genotypes are ideal for initiating breeding program for the species. Despite showing little improvement, cowpea has genetic variability in some variables of agronomic interest, highlighting the potential of each population for selection (Correa et al., 2015).

Some genotypes showed similar performance and were clustered in small groups. Genotypes CE-994, 999, 1002, and 1007 and the cultivar BRS-Guariba (CE-934) strongly correlated with the phenotypic variables under evaluation (Fig. 2).

Genotypes CE-164, 207, and the cultivars BRS-Paraguaçu (938) and BRS-Tumucumaque (97) formed another group. Six genotypes, CE-685, 689, 243, 688, 155, and 12 showed a strong correlation. In addition to these groups, some pairs of genotypes were similar for quantitative and qualitative variables, e.g., CE-248 and 18, 70 and 25, 686 and 17, and CE-33 and 5.

Even with overlapping lines (genotypes), some genotypes showed greater genetic divergence (less similarity), e.g., CE-165, whose variables differed from the others for green-bean production, showing large (35.42 cm) and wide pods (0.83 cm) and being a late genotype, with 59 days to the harvest of green pods.

Other genotypes were also divergent, e.g., CE-98, 244, 22, 96, and 68. CE-98 showed 69 days to harvest and large pods (1.08 cm), with a pod length of 20.58 cm. These genotypes showed a different behavior for the evaluated variables, especially those with high heritability values, and are important for identifying genetic diversity among genotypes. The success of a breeding program depends mainly on the availability of populations with high genetic variability for the traits under selection (Azevedo et al., 2013).

Similarity studies are common in genotype grouping. However, there are no reports of correlation or distance studies that can help to classify cowpea genotypes using similarity networks, this being the first such study. Other studies have been carried out with various crops for different purposes, e.g., correlating phenotypic and genotypic variables in pepper plants (Silva et al., 2016) and studying adaptability and stability in rice (Silva et al., 2020), both cases proving to be efficient.

Table 1. Estimates of the relative contribution of each variable to genetic diversity among cowpea genotypes.

Variable	Relative contribution of each variable (%)
Green-pod thickness	18.40
Green-pod width	16.20
Days to fruiting	14.10
Green-pod length	11.50
Green-grain thickness	6.10
Grain weight per green pod	5.10
Plant height	4.40
Number of nodes on the main branch	4.20
Days to flowering	4.00
Green-pod weight	4.00
Stem diameter	3.60
Green-grain length	3.00
Number of loculi per pod	2.00
Number of grains per pod	2.00
Green-grain width	0.50

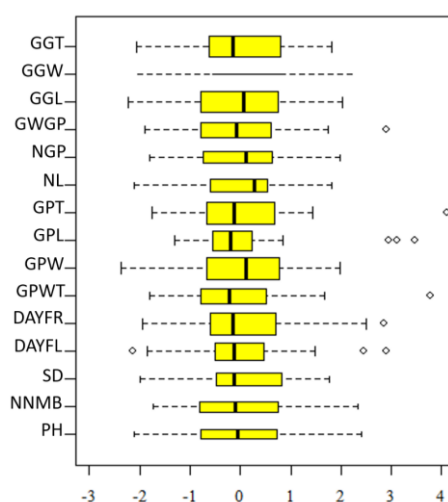


Fig 1. Boxplots of morpho-agronomic variables in 44 cowpea genotypes standardized for a mean value of 0 and variance of 1. Plant height (PH), days to flowering (DAYFL), days to fruiting (DAYFR), stem diameter (SD), number of nodes on the main branch (NNMB), green-pod length (GPL), green-pod weight (GPWt), number of loculi per pod (NL), green-pod width (GPW), green-pod thickness (GPT), green-grain thickness (GGT), green-grain width (GGW), grain weight per green pod (GWGP), number of grains per pod (NGP), and green-grain length (GGL).

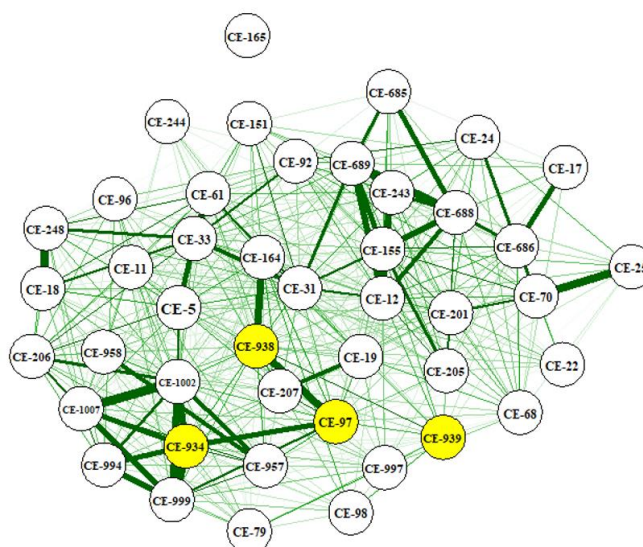


Fig. 2. Similarity network of cowpea genotypes based on the conjugate distance matrix using the Mahalanobis distance for numerical variables and simple index of coincidence for categorical variables. Connections representing a similarity greater than 70% are highlighted, and only connections greater than 50% similarity are shown.

Materials and Methods

Experimental site and plant material

The rainfed experiment was carried out in the experimental area of the horticultural sector at the Plant Science Department of the Agricultural Sciences Center of the Federal University of Ceará (CCA/UFC), Pici Campus (3°44'24.4" S and 38°34'32.0" W) in Fortaleza, Ceará, Brazil. According to data from the on-campus weather station during the experimental period, the accumulated rainfall was from February to May 2020, 111.8 mm, whereas the mean temperature was 27.3°C.

Treatments consisted of 38 cowpea (*V. unguiculata*) genotypes of unknown genetic potential (CE-24, 61, 68, 70, 114, 123, 151, 164, 165, 172, 189, 199, 201, 205, 206, 207, 228, 243, 244, 248, 253, 313, 337, 542, 685, 686, 688, 689, 925, 957, 958, 964, 986, 994, 997, 999, 1002, and 1007) and four commercial cultivars: BRS Guariba (934), BRS Tumucumaque (97), BRS Paraguaçu (938), and Paulistinha (939), all belonging to the Active Germplasm Bank (AGB) of the CCA/UFC Plant Science Department.

Conduction of the study

The working area of each block measured 52.5 m² and was formed by five central rows 10.5 m long and 1.0 m wide. Two rows were adopted as borders on each side, and the spacing was 1.0 m between rows and 0.50 m between plants in the row.

Three seeds were sown per hole, and the plants were thinned to two plants per hole 15 days after sowing. The soil of experimental area was prepared conventionally by plowing and harrowing. Fertilization was based on a chemical soil analysis and considering the crop requirements. Crop management practices consisted of hoeing, which was performed for weed control during seedling emergence and close to flowering. In addition, insecticides were applied for pest control during crop development.

Variables collected

Fifteen quantitative variables were considered for the morpho-agronomic characterization: plant height (PH), days to flowering (DAYFL), days to fruiting (DAYFR), stem diameter (SD), number of nodes on the main branch (NNMB), green-pod length (GPL), green-pod weight (GPWt), number of loculi per pod (NL), green-pod width (GPW), green-pod thickness (GPT), green-grain thickness (GGT), grain weight per green pod (GWGP), number of grains per pod (NGP), and green-grain length (GGL), in addition to seven qualitative variables: growth (GR), plant anthocyanins (PAN), flower color (FLCO), plant habit (PHb), mature pod color (MPC), mature pod pigmentation (MPP), green-grain width (GGW), and seed color (SC).

Statistical analysis

The residual variance-covariance matrix was obtained by subjecting the numerical data to a multivariate analysis of variance based on an augmented block design with common treatments, with the genotypes being repeated six times within the block. The vectors of mean genotypic values were corrected according to Pimentel-Gomes (2009) by subtracting the effects vector from the respective block.

The adjusted mean vectors were used to obtain the generalized Mahalanobis distance matrix, based on which the relative importance of the variables was calculated using

the method proposed by Singh (1981). A distance matrix was also obtained based on categorical variables using the simple index of coincidence according to Cruz et al. (2011). Both matrices were rescaled [0,1] to obtain a conjugate distance matrix based on the mean distance of the pairs. Heritability was estimated to identify variables with high genotype discrimination capacity and guarantee genetic predictability when recommending these variables for genotype selection. The conjugate distance matrix was graphically represented using similarity networks, as demonstrated by Silva et al. (2016) and Silva et al. (2020), aiming to identify patterns of similarity in the matrix and study phenotypic divergence. Statistical analysis was performed using the R v3.5.3 software (www.r-project.org) with the biotools (Silva, 2017) and qgraph (Epskamp et al., 2012) packages.

Conclusion

The CE-164, 207, 999, 994, 1002, and 1007 genotypes should be avoided in breeding programs aimed at green-pod production since they show genetic similarity with the commercial cultivars BRS Guariba, BRS Tumucumaque, and BRS Paraguaçu. The variables of days to fruiting, green-pod length, green-pod width, green-pod thickness, and green-grain thickness contribute to genetic divergence and have high heritability values. There is genetic variability among cowpea genotypes meant for green-bean production. Crosses between cowpea genotypes CE-165, 244, 22, 96, and 98 can yield gains in green-grain production in advanced generations.

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