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Prospection of cowpea genotypes for green-grain production[1]

Prospecção de genótipos de feijão-caupi para a produção de grãos verdes

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ABSTRACT - Cowpea is a nutritious species cultivated worldwide whose high genetic variability can be exploited in breeding programs. The present study aimed to identify phenotypically divergent genotypes of *Vigna unguiculata* (L.) Walp., from this perspective concerning agronomic and physiological variables aiming at green grain production. An experiment was conducted in Fortaleza – CE, to analyse 13 variables in 44 genotypes from the Active Germplasm Bank of the Federal University of Ceará. The experiment was set up in an augmented block design with four additional controls (commercial cultivars). The genetic and environmental variance components were estimated by restricted maximum likelihood, after which the intraclass correlation coefficient (ICC) was calculated. Principal components analysis, Tocher's clustering and correlation analysis were used in the study. The variables most influenced by genetic variance were production per plant, green pod length, green grain length, grain mass per pod, and total yield, with ICC > 0.5. Principal components analysis signalled the physiological variables gs, ci, and ci.ca and the agronomic variables of green pod length, green grain mass, number of grains per pod, and earliness of production as important for differentiating between cowpea genotypes. CE genotypes 02, 151, 165, 172, 189, 244, 986, and 1002 show genetic variability, and their use is recommended in cowpea breeding programs aimed at green-grain production.

Key words: Phenotypic characteristics. Intraclass correlation. Parental selection. Genetic variation. *Vigna unguiculata*.

RESUMO - Feijão-caupi é uma espécie nutritiva, cultivada em várias partes do mundo, com ampla variabilidade genética que pode ser explorada no melhoramento. Este trabalho teve como objetivo identificar genótipos fenotipicamente divergentes de *Vigna unguiculata* (L.) Walp., sob esta perspectiva quanto as variáveis agronômicas e fisiológicas visando produção de grãos verdes. Em um experimento conduzido em Fortaleza - CE, 13 variáveis foram avaliadas em 44 genótipos provenientes do Banco Ativo de Germoplasma da Universidade Federal do Ceará. O experimento foi instalado em delineamentos de blocos aumentados, com quatro testemunhas adicionais (cultivares comerciais). Os componentes de variância genético e ambiental foram estimados por máxima verossimilhança restrita e, a partir destes, foi calculado o coeficiente de correlação intraclasse (ICC). Foi realizada análise de componentes principais, agrupamento de Tocher e análise de correlação. As variáveis mais influenciadas pela variação genética foram: produção por planta, comprimento da vagem verde, comprimento do grão verde, massa de grãos por vagem e produtividade total, com ICC > 0.5. A análise de componentes principais permitiu identificar as características fisiológicas: gs, ci, ci.ca, e agronômicas: comprimento da vagem verde, massa de grãos verdes, número de grãos por vagem e precocidade da produção, como sendo importantes na diferenciação de genótipos de feijão-caupi. Os genótipos CE: 02, 151, 165, 172, 189, 244, 986 e 1002 possuem variabilidade genética e são recomendados para uso em um programa de melhoramento genético de feijão-caupi para produção de grãos verdes.

Palavras-chave: Características fenotípicas. Correlação intraclasse. Seleção de parentais. Variação genética. *Vigna unguiculata*.

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INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is a plant species from Africa. It is one of the main tropical grain legumes, with excellent nutritional and nutraceutical properties (BRILHANTE *et al*., 2021). Its economic importance is highlighted in many African countries (ROCHA *et al*., 2021) and intertropical America, especially in the semi-arid region of northeastern Brazil. Furthermore, its green pods are considered one of the top ten vegetables consumed in Asia (PASQUET; FELEKE; GEPTS, 2021). In Brazil, cowpea is known by several common names, such as feijão-frade, feijão-de-corda, feijão-de-praia, and feijão-macassar (DINGHA *et al*., 2021; MARINHO *et al*., 2021; PÚBLIO JÚNIOR *et al*., 2018).

The high genetic variability of this species is a determining factor in its production potential. Some individuals have allelic variations that provide adaptation to a particular condition. Such characteristic can be used to improve other genotypes by selection and artificial reproduction (NKOMO; SEDIBE; MOFOKENG, 2021), resulting in more productive, biofortified, new genotypes adapted to different environments and production systems (SILVA *et al*., 2018).

From this perspective, studies on genetic dissimilarity and estimation of genetic parameters are essential to guide selection strategies. For that purpose, it is essential to know the genetic variability of germplasm collections as the exploration of this basic material allows finding genotypes with the potential for breeding programs.

Several studies have been performed on the genetic divergence of cowpea aiming at genotype selection. Barroso Neto *et al*. (2017), Santana *et al*. (2019) and Sousa *et al*. (2017) assessed the genetic divergence of cowpea genotypes and suggested combinations to obtain promising populations. The present study aimed to identify phenotypically divergent genotypes of *V. unguiculata*, from this perspective concerning agronomic and physiological variables aiming at green grain production.

MATERIAL AND METHODS

The experiment was conducted in an experimental area belonging to the Horticulture Sector of the Plant Science Department of the Center of Agricultural Sciences of the Federal University of Ceará (CCA/UFC), Campus Pici (3º44'24.4" S; 38º34'32.0" W; elevation of 19.5 m), Fortaleza, Ceará, Brazil, from August to November 2020. According to the Köppen classification, the climate of the area is classified as Aw or rainy tropical.

Treatments consisted of 44 cowpea genotypes: CE 02, 24, 61, 68, 70, 114, 123, 151, 155, 164, 165, 172, 189, 199, 201, 205, 206, 207, 228, 243, 244, 248, 253, 313, 337, 398, 542, 685, 686, 688, 689, 925, 957, 958, 964, 986, 997, 999, 1002, 1007, and four commercial cultivars: BRS Guariba, BRS Tumucumaque, BRS Juruá and BRS Aracê, all belonging to the Active Cowpea Germplasm Bank (BAG) of the Plant Science Department of CCA/UFC.

The total area of the experiment was 214 m^2. Each 52 m^2 block was formed by five central rows of 13 m in length, one meter between lines, one meter between genotypes in the line and two meters wide between the blocks. The spacing adopted was 1.0 m between rows and 0.50 m between plants in the row, and each genotype was composed of six plants.

Three seeds were sown per hole, and plants were thinned to two plants per hole 15 days after sowing. Soil preparation was based conventionally by plowing and harrowing. Fertilization was performed based on the crop requirements and soil chemical analysis. Crop management consisted of weed control by hoeing during seedling emergence and close to flowering, and insecticides were applied for pest control during crop development. A localized micro-sprinkler irrigation system was used, and plants were irrigated once a day early in the morning to keep the soil between 70 and 90% of field capacity.

The following agronomic variables were evaluated: earliness of production [(EP) determined by counting the days from sowing to the beginning of harvest in each treatment]; production per plant [(PP - g/plant) determined by individually weighing and summing the pods from each plant]; green pod length [(GPL - cm) determined as the mean of ten pods per plant with the aid of a ruler]; number of pods per plant [(NPP) determined by counting the number of pods from each plant]; green grain length [(GGL - cm) determined with the aid of a digital caliper based on 10 grains], grain mass per pod [(GMP -g) determined by weighing the grains from each pod and considering the mean of 10 pods], and total yield [(PY - t/ha) determined by summing the yield of marketable grains].

Gas exchange was evaluated at the beginning of the reproductive phase, between seven and eleven o'clock in the morning, with the aid of a portable infrared gas analyzer (ADC® LCi). The following parameters were evaluated: internal CO_2 concentration (Ic, ppm); stomatal conductance (Sc, $\mu mol.m^{-2}.s^{-1}$); net photosynthesis (P, $\mu mol.m^{-2}.s^{-1}$); the ratio between the CO_2 concentration in the substomatal chamber and the CO_2 concentration in the environment (Ic.Sc), and instantaneous carboxylation efficiency (E), obtained as the ratio between photosynthesis (P) and Ic.

A mixed linear model was adjusted for each response variable by considering the genotype effect as random. The genetic and residual (environmental) variance components were estimated by restricted maximum likelihood (REML). Based on these, it was possible to calculate the intraclass correlation coefficient (ICC) $ICC = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_E^2}$ representing the proportion of genetic variation. The random genotype effects were also considered (BLUPs – best linear unbiased predictors), being used in the principal components analysis, whose two first components were shown in a biplot graph containing 95% confidence ellipses for the groups of genotypes and cultivars. Also based on the BLUPs, Tocher's clustering was performed based on the distance matrix of Mahalanobis.

Residual correlations were subjected to Student's t-test at 5% significance, and the correlation matrix was presented graphically.

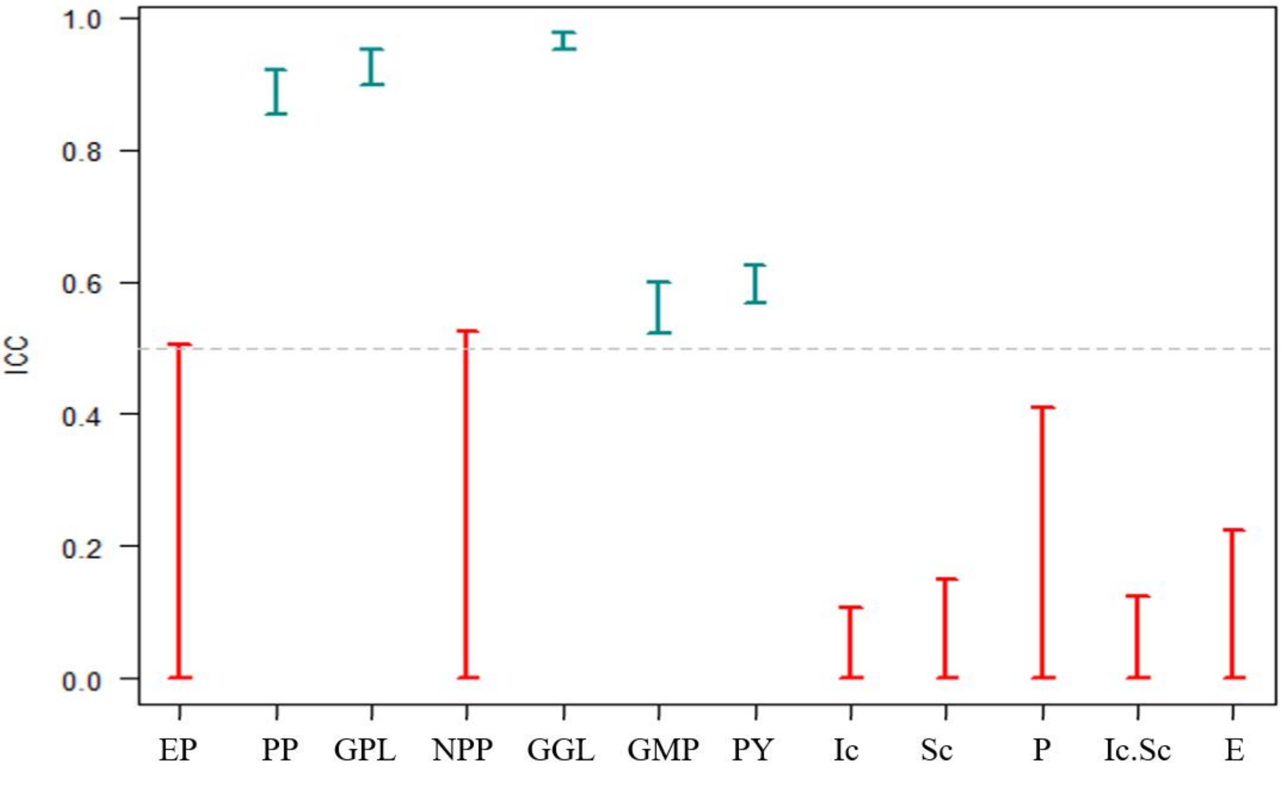
The analyses were performed with the statistical packages *nlme* (PINHEIRO *et al*., 2018), *biotools* (SILVA, 2020) and *corrplot* (WEI; SIMKO, 2017).

RESULTS AND DISCUSSION

Significant values were observed ($p < 0.05$) for the intraclass correlation coefficient (ICC), above 0.5, for the agronomic variables of production per plant (PP), green pod length (GPL), green grain length (GGL), grain mass per pod (GMP), and total yield (PY) (Figure 1), suggesting a high proportion of genetic variation. This coefficient represents a trend toward the maintenance of phenotypic superiority of the genotypes in the evaluated environment, and the higher the correlation, the lower the interference of the genotype x environment interaction (LAVIOLA *et al*., 2014). This index has been considered appropriate to assess the consistency and conformity of studies as it allows estimating the proportion of total variation due to variability between the independent units of analysis (AGUIAR; FONSECA; VALENTE, 2010).

The physiological variables, on the other hand, showed low values (< 0.5) of intraclass correlation (non-significant, $p > 0.05$), as well as the agronomic variables of earliness of production (EP) and number of grains per pod (NPP). Low ICC values were expected as these variables are more influenced by uncontrollable, environmental aspects

Figure 1 - 95% confidence intervals for the intraclass correlation (ICC) of agronomic and physiological variables of cowpea. Earliness of production - EP, production per plant - PP, green pod length – GPL, number of pods per plant - NPP, green grain length - GGL, grain mass per pod - GMP, total yield - PY, internal CO_2 concentration - Ic, stomatal conductance - Sc, net photosynthesis - P, ratio between the CO_2 concentration in the substomatal chamber and the CO_2 concentration in the environment – Ic.Sc, and instantaneous carboxylation efficiency - E



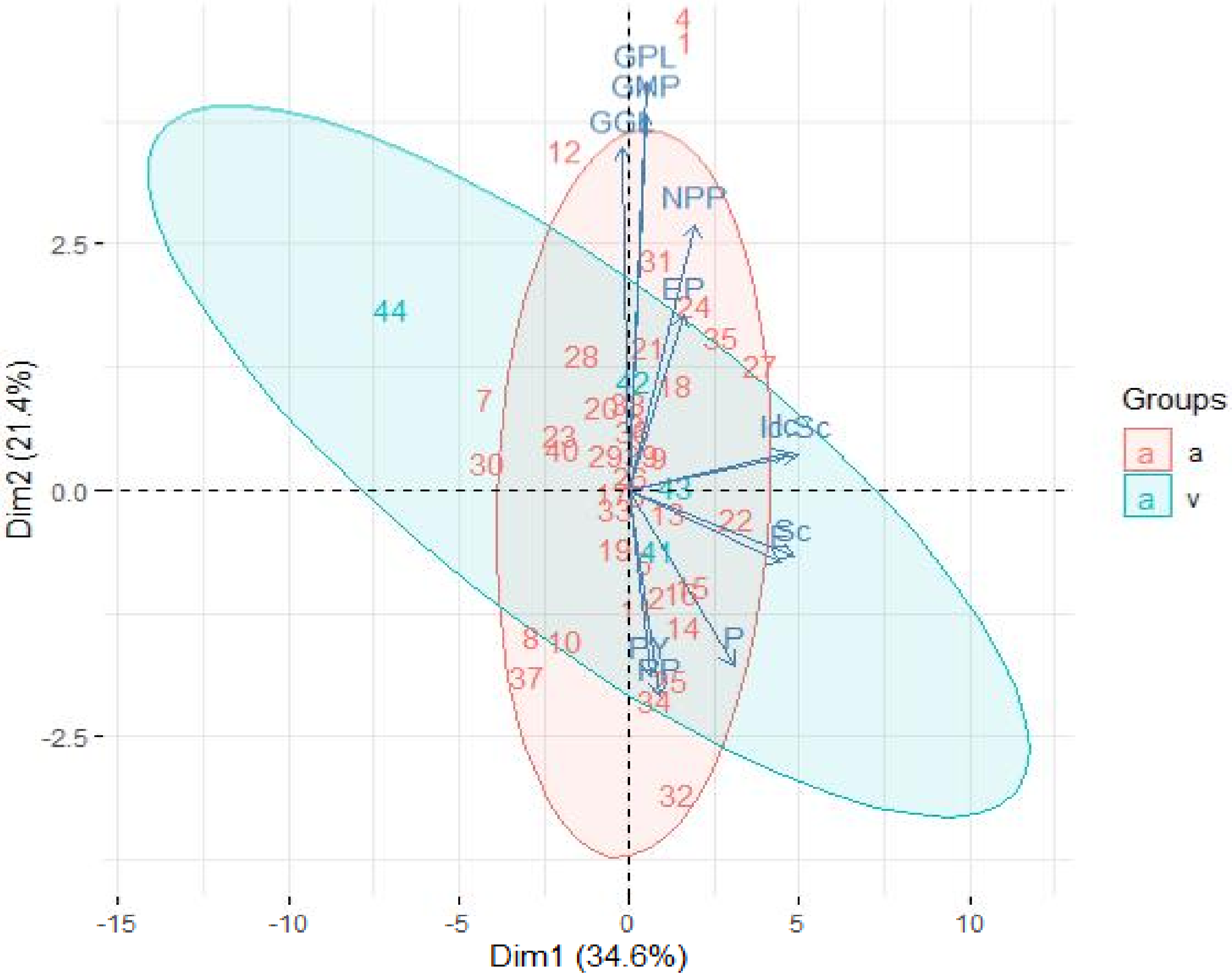
that interfere with selection studies, such as light, which interferes with the processes of enzymatic activation, stomatal opening and closing, and the photosynthetic activity, among others, influencing plant growth and development (COELHO *et al*., 2014).

For the agronomic variables, Oliveira *et al.* (2017) reported low heritability values for the earliness of production and the number of grains per pod in cowpea, highlighting the greater influence of the environment on the phenotypic expression of genetic materials with regard to these variables (YOKOMIZO *et al*., 2021), being less suitable for selection.

The reduction in the number of characteristics for two principal components facilitated the genetic divergence assessment of cowpea genotypes based on genetic values (BLUPs) for the studied variables (Figure 2). The influence on genotype performance could be graphically highlighted regardless of the species evaluated (FARIAS NETO *et al*., 2018).

In the biplot, the groups of variables coincided with two clear types of genetic variation: 1) physiological variation, related to CP1, and 2) agronomic variation, related to CP2. The first two components explained 56.0%

Figure 2 - BLUPs biplot of 44 cowpea genotypes [1(02), 2(24), 3(155), 4(172), 5(199), 6(206), 7(313), 8(542), 9 (689), 10(1002), 11(61), 12(151), 13(164), 14(205), 15(243), 16(244), 17(337), 18(686), 19(964), 20(986), 21(68), 22(114), 23(123), 24(165), 25(201), 26(228), 27(253), 28(685), 29(997), 30(999), 31(70), 32(189), 33(207), 34(248), 35(688), 36(925), 37(957), 38(958), 39(398), 40(1007), 41(BRS Aracê), 42(BRS Guariba), 43(BRS Juruá), and 44(BRS Tumucumaque)] based on agronomic and physiological variables, and 95% confidence ellipses for the means of groups, accessions (a), and variety (v). Earliness of production - EP, production per plant - PP, green pod length – GPL, number of pods per plant - NPP, green grain length - GGL, grain mass per pod - GMP, total yield - PY, internal CO_2 concentration - Ic, stomatal conductance - Sc, net photosynthesis - P, ratio between the CO_2 concentration in the substomatal chamber and the CO_2 concentration in the environment – Ic.Sc, and instantaneous carboxylation efficiency - E



of the variability of genetic values (Figure 2), with 34.6% being retained in CP1, whose higher loads were gs, ci, and Ca, with greater ability to separate genotypes. Genotype 44 (BRS Tumucumaque) stood out with lower overall photosynthetic activity.

PC2, with greater weights of the variables of green pod length (GPL), green grain length (GGL), and grain mass per pod (GMP), comprised 21.4% of the total variance, especially genotypes 1(02), 4(172), and 12(151) (Figure 2).

The dispersion of genotypes as a function of the first two components suggests that individuals 1 (02), 4 (172), 7 (313), 12 (151), 22 (114), 27 (253), 30 (999), 32 (189) 37 (957), and cultivar 44 (BRS Tumucumaque) (Figure 2) stood out among the evaluated genotypes, diverging with regard to agronomic and physiological variables for being more dispersed from the origin of the Cartesian plane. Santos *et al.* (2016) also selected the best cowpea genotypes by principal components analysis using biplot graphs. This analysis effectively determines the proximity and distance of a genotype from the others (OLIVEIRA *et al*., 2019).

Clustering analysis by Tocher's method separated the 44 genotypes into six different groups (Table 1). This information is important when choosing parents to initiate a breeding program.

Thirty-two genotypes formed group I, composed of 28 genotypes (986, 68, 155, 207, 123, 337, 353, 688, 685, 70, 689, 964, 398, 28, 925, 999, 1007, 114, 243, 206, 201, 997, 61, 686, 24, 199, 452, and 244) and four commercial cultivars (BRS Juruá, BRS Guariba, BRS Aracê, and BRS Tumucumaque), corresponding to 81.82% of the genotypes evaluated (Table 1). The agronomic and physiological variables of the genotypes some features similar to the cultivars, being ideal for selection for showing interesting production aspects as the individuals within the group are more similar than the individuals between groups.

Group II was formed by four genotypes (1002, 958, 957, and 165) which had some similar characters, corresponding to 9.10% of the total individuals evaluated. Groups III, IV, V, and VI were formed by one genotype each, 02 (2.27%), 172 (2.27%), 151 (2.27%) and 189 (2.27%), respectively. The genotypes with genetic variation found in this study are favorable resources for breeding programs.

Differences in-group composition by Tocher's method are important when choosing parents and recommending the heterotic potential (SANT'ANNA *et al*., 2021) and when segregating populations for later selection. Once these divergent genotypes are identified, it is also important to confirm them according to agronomic variables of interest and the selection objectives.

Genetic divergence among 30 cowpea genotypes were observed based on the formation of three different groups by the Tocher's optimization method (SANTANA *et al*., 2019). Dissimilar genotypes from different groups are recommended for selection in order to generate promising populations (SOUSA *et al*., 2017). Thus, a greater potential for selection should be attributed to genotypes 986, 244, 1002, 165, 02, 172, 151, and 189 in order to develop cowpea cultivars aimed at green-grain production.

The principal components method and Tocher's clustering agreed on the discrimination of some cowpea genotypes, CE 02, 151, 172, and 189.

Pearson's correlation coefficients are represented in the heat map shown in Figure 3. Some correlations were observed between significant agronomic and physiological variables ($p < 0.05$). The study of correlations generates data that result in greater or lesser difficulty in choosing genotypes with the desirable characteristics for the breeding program (DALCHIAVON *et al*., 2016).

The variables of earliness of production (EP) x green grain length (GGL) (-0.76), green grain length (GGL) x internal CO_2 concentration (Ic) (-0.76), and green grain length (GGL) x the ratio between the CO_2

Table 1 - Composition of the groups of cowpea genotypes based on the clustering analysis by Tocher's optimization method based on the distance matrix of Mahalanobis

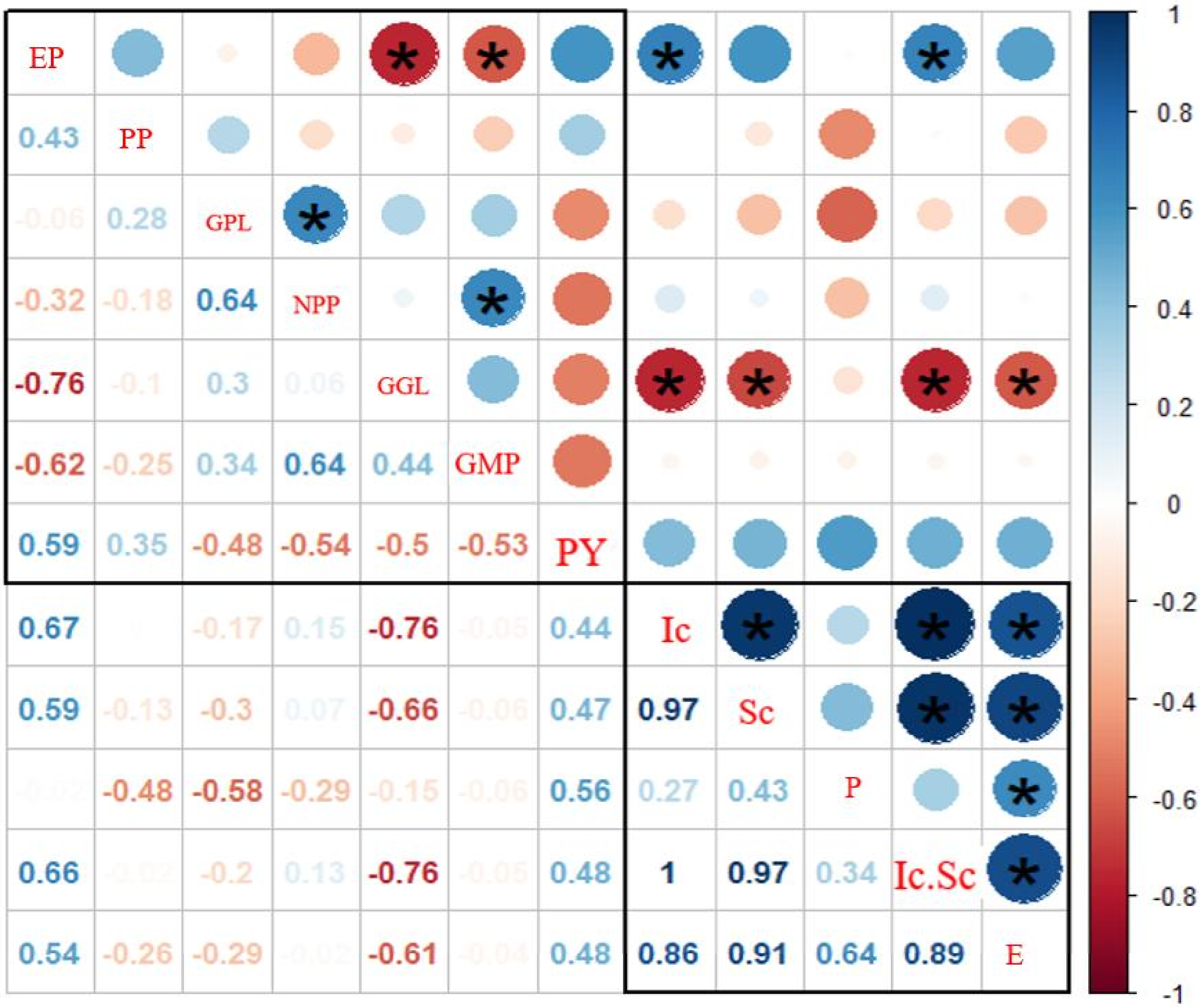
Groups	Genotypes
I	986, 68, 155, 207, 123, 337, 353, 688, 685, 70, 689, 964, 398, 28, 313, 205, 164, 248, BRS Juruá, 925, 999, 1007, 114, BRS Guariba, 243, 206, 201, 997, 61, 686, 24, BRS Aracê, 199, BRS Tumucumaque, 452, and 244.
II	1002, 958, 957, and 165
III	02
IV	172
V	151
VI	189

concentration in the substomatal chamber and the CO_2 concentration in the environment (Ic.Sc) (-0.76) showed significant and negative correlations (Figure 3), evidencing that, when the values of one variable increased, the values of the other decreased. Although negatively correlated, these variables were classified as of moderate magnitude. Negative correlation estimates between pairs of variables indicate that the improvement of one character will decrease the other, and selection based on one of them is not recommended (CORREA *et al*., 2015).

The magnitude of the correlation between two or more variables assumes values from -1 to +1, which means that positive coefficients indicate a direct correlation between variables, while negative coefficients imply an inverse correlation (ALMEIDA *et al*., 2020).

Green grain length (GGL) showed a negative correlation with three variables, suggesting that this variable had a negative influence and should be considered in cowpea breeding programs aimed at green-grain production. When studying cowpea, Alves *et al.* (2020) also reported a significant correlation between pod and grain variables, suggesting the possibility of associating these characteristics with implications for selection.

Figure 3 - Residual correlations between agronomic and physiological variables of cowpea. The symbol * over correlation circles indicate a significant correlation ($p < 0.05$). Earliness of production - EP, production per plant - PP, green pod length - GPL, number of pods per plant - NPP, green grain length - GGL, grain mass per pod - GMP, total yield - PY, internal CO_2 concentration - Ic, stomatal conductance - Sc, net photosynthesis - P, ratio between the CO_2 concentration in the substomatal chamber and the CO_2 concentration in the environment – Ic.Sc, and instantaneous carboxylation efficiency - E



A significant, positive, and high correlation was verified between the physiological variables of internal CO_2 concentration (Ic) x stomatal conductance (Sc) (0.97), internal CO_2 concentration (Ic) x transpiration (E) (0.86), stomatal conductance (Sc) x ratio between the CO2 concentration in the substomatal chamber and the CO2 concentration in the environment (Ic.Sc) (0.97), stomatal conductance (Sc) x transpiration (E) (0.91), and the ratio between the CO_2 concentration in the substomatal chamber and the CO_2 concentration in the environment (Ic.Sc) x transpiration (E) (0.89). These correlations were surely expected as they are related to physiological aspects of the plant, acting dependently. When the correlations are high and positive, these variables reveal that a character behaves to the detriment of the other, with the same physiological processes, facilitating indirect selection (CORREA *et al*., 2015).

CONCLUSIONS

1. Principal components analysis based on BLUPs allowed identifying the physiological variables gs, ci, and Ci.ca, and the agronomic variables of green pod length, green grain mass, number of grains per pod, and earliness of production as important for the differentiation of cowpea genotypes. However, a higher proportion of genetic variation was observed for the variables of green pod length, green grain length and production per plant;
2. There is greater genetic variability in genotypes CE 02, 151, 165, 172, 189, 244, 986, and 1002, which are recommended for use in cowpea breeding programs aimed at green-grain production.

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