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Recommendation of cowpea genotypes based on adaptability, stability and grain darkening[1]

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ABSTRACT - The cowpea is a legume that is widely grown in the north-east of Brazil, and which has been gaining ground in other regions of the country. The main producer is the state of Ceará, with a large planted-area, albeit low productivity due to a lack of producer technology and adapted cultivars. The aim of this study was to identify and recommend superior genotypes in terms of adaptability and stability under rainfed and irrigated conditions, in addition to genotypes with reduced grain darkening. To this end, six experiments were conducted in different districts of Ceará (Crato, Pentecoste, Crateús, Madalena, Bela Cruz and Limoeiro do Norte) and one laboratory experiment, to evaluate grain darkening. The experimental design of the field trials was of randomised blocks, with 14 genotypes and 4 replications. The analysis of variance showed a significant effect from the genotypes and environments and their interaction, so GGE Biplot analysis was carried out to evaluate adaptability and stability. To evaluate grain darkening, a completely randomised design was used in a simple factorial scheme with six previously selected genotypes and five different storage times (0, 2, 4, 6 and 8 months). There was a significant effect from the genotypes and storage time. Genotype 1 showed the least darkening, and can be recommended for environments to which it is best adapted (Crato and Crateús). Genotype 9 was considered the most stable for grain yield, and can be more broadly recommended for the semi-arid region of the state of Ceará.

Key words: *Vigna unguiculata*. GGE Biplot. Genotype x environment interaction.

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INTRODUCTION

The cowpea [*Vigna unguiculata* (L.) Walp.] is a legume with a wide global distribution, and is of great importance in semi-arid regions. It originated on the African continent, which is its largest producer, especially Nigeria, Niger and Burkina Faso (Omomowo; Babalola; Oluranti, 2021). The species is also grown in other regions, such as Southwest Asia, the Mediterranean Basin, the United States and Latin America, particularly Brazil (Herniter; Muñoz-Amatriaín; Close, 2020).

Cowpea cultivation includes various methods, ranging from rainfed subsistence, practised by farmers with little access to technologies such as additives and improved seeds, to highly technological systems (Herniter; Muñoz-Amatriaín; Close, 2020; Kebede; Bekeko, 2020). As a result, the average crop yield is well below potential in the major growing areas (Abiriga *et al.*, 2020; Araméndis-Tatis; Cardona-Ayala; Espitia-Camacho, 2021). In Brazil, the species is mainly grown in the north-east of the country, which, due to the large planted area, is the largest producer, despite the yields being low (EMBRAPA, 2020).

Some cowpea varieties exhibit darkening of the seed coat during storage, resulting in losses due to a reduction in their commercial value (Lima; Tomé; Abreu, 2014). The light colour of the seed coat is usually associated with the characteristics of freshly harvested beans (Ribeiro; Jost; Cargnelutti Filho, 2004), with darker beans associated with older beans that are difficult to cook. In the north-east, in particular, there is a preference for mulatto seeds; however, these tend to suffer from darkening, making it necessary to recommend genotypes for the region that not only meet the needs of the market but also show less darkening. It is widely recognised that there is still a shortage of technologies aimed at breeding cultivars that combine desirable phenotypes, such as resistance to biotic and abiotic stress, suitable architecture, commercially accepted grain, and high productivity (Alves *et al.*, 2020).

To safely recommend new genotypes, it is essential to understand the interaction between the genotype and the environment, for which it is necessary to evaluate the genetic materials in different locations and/or at different planting times (Abiriga *et al.*, 2020; Abreu *et al.*, 2019; Araméndis-Tatis; Cardona-Ayala; Espitia-Camacho, 2021). Furthermore, the type of cultivation system adopted, i.e. rainfed or irrigated, must be taken into account, as each provides the genotypes with different environmental conditions, while the yield of the species is highly influenced by the water regime (ĎZdemir; Ünlükara; Kurunc, 2009). To obtain information about the behaviour of the genotypes in each environment and continue to meet market demand, it is essential to select strains that are highly adaptable and stable in the main production areas and principal cultivation systems (Alves *et al.*, 2020; Cruz *et al.*, 2021).

The GGE Biplot method is based on principal component analysis, and is widely used to estimate adaptability and stability (Cruz *et al.*, 2020). In the GGE Biplot method, the effect of a genotype (G) is obtained as a multiplicative effect of the genotype x environment interaction (GE), remembering that the isolated environmental effect is not suitable for recommending genotypes (Abreu *et al.*, 2019). The aim of this study was to analyse the interaction between genotypes and environments, and select cowpea genotypes of greater productivity, stability and adaptability, and with less grain darkening, for irrigated and rainfed cultivation systems in the semi-arid region of Ceará.

MATERIAL AND METHODS

Experimental design

Six Value for Cultivation and Use (VCU) trials were conducted on 14 cowpea genotypes from the Cowpea Improvement Program of Embrapa Meio-Norte. The genotypes consisted of twelve strains obtained from the selection of individual plants with progeny testing; two cultivars were used as controls (Table 1).

The trials were conducted in various locations in the state of Ceará, in the districts of Crato, Pentecoste, Crateús, Madalena, Bela Cruz and Limoeiro do Norte, located in five mesoregions of the state (Figure 1). The experiments were set up at different times of the year. Experiments E1, E3 and E5 were irrigated, while the other trials were rainfed during the rainy season (Table 2).

A randomised block design (RBD) was used, with four replications. Each experimental plot was 10 m², comprising four rows, each 5 m in length and spaced 0.5 m apart. The central rows were evaluated, with the two side rows representing the border. The spacing between each hole was 0.25 m, with two plants per hole to give a population of 160 thousand plants ha^{-1}.

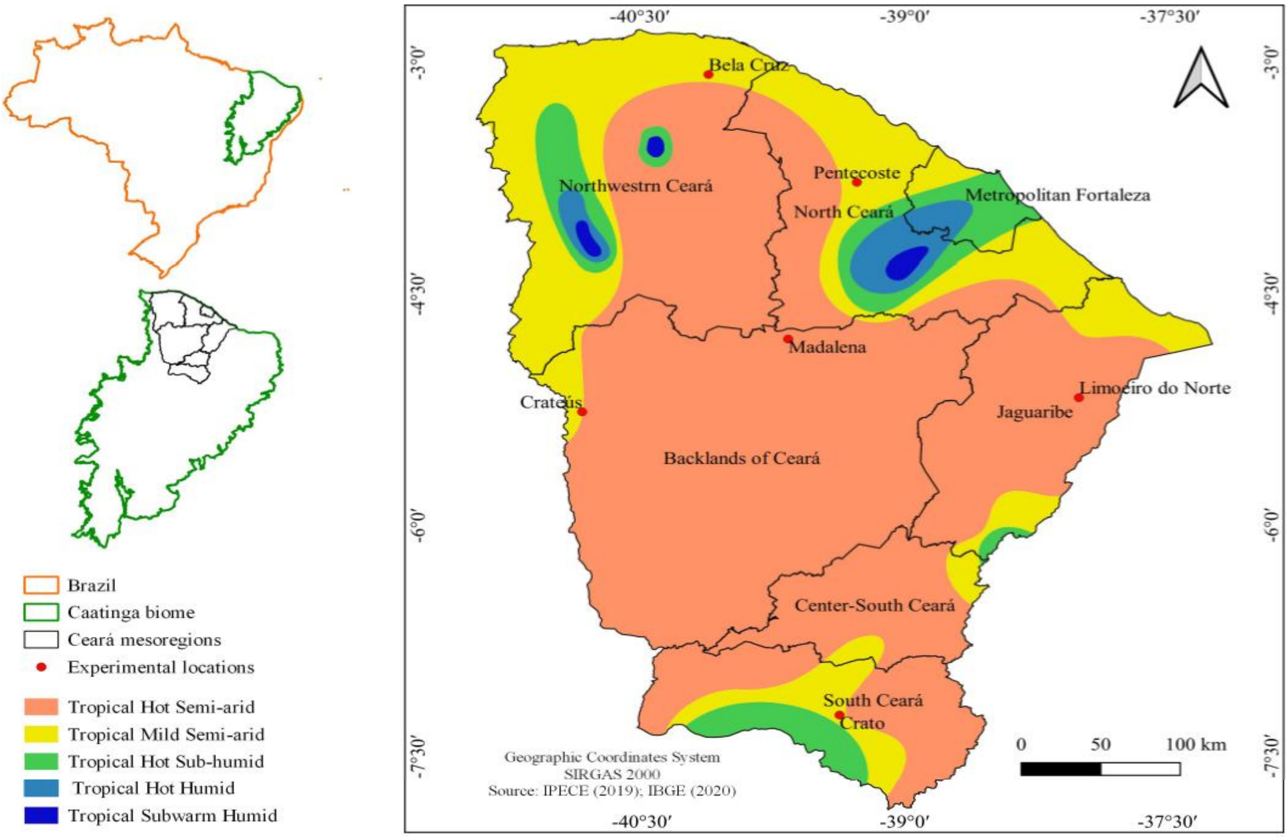
The soil in each area was prepared conventionally by ploughing and harrowing. Fertilisation was carried out in accordance with the soil analysis (Appendix A) and crop recommendations (Cravo; Viégas; Brasil, 2007). Single superphosphate and potassium chloride fertilisers were used when planting. A top dressing of urea was used as a source of nitrogen 15 days after planting. Deltamethrin- and sulphur-based pesticides were used to control pests. Grain productivity (PROD) in kg ha^{-1} was evaluated in the different trials.

Table 1 - Identification, origin and commercial subclass of the genotypes used in the study

ID	Genotype	Parents/Origin	Commercial Subclass
1	Bico-de-ouro 1-5-11	Selection of individual plants with progeny testing, from plants collected in the state of Mato Grosso	SV
2	Bico-de-ouro 1-5-15		SV
3	Bico-de-ouro 1-5-19		SV
4	Bico-de-ouro 1-5-24		ML
5	Pingo-de-ouro 1-5-26	Selection of individual plants with progeny testing, from plants collected in the semi-arid region of the state of Piauí	ML
6	Pingo-de-ouro 1-5-4		ML
7	Pingo-de-ouro 1-5- 5		ML
8	Pingo-de-ouro 1-5-7		ML
9	Pingo-de-ouro 1-5-8		ML
10	Pingo-de-ouro 1-5-10		ML
11	Pingo-de-ouro 1-5-11		ML
12	Pingo-de-ouro 1-5-14		ML
13	BRS Tumucumaque	TE96-282-22G x IT87D-611-3	BR
14	BRS Imponente	MNC00-553D-8-1-2-3 x MNC01-626F-11-1	BC

Source: prepared by the author. ID: Identification; BC: ´Brancão´; BR: ´Branco´; ML: ´Mulato´; SV: ´Sempre-verde´

Figure 1 - Locations of the value for cultivation and use (VCU) trials in the semi-arid region of the state of Ceará



Source: prepared by the author

Table 2 - Environments of the trials and their respective codes, sowing date, altitude, geographic coordinates, and rainfall accumulated during the tests

Code	Locality	Sowing	Altitude	Latitude	Longitude	Rainfall
E1	Crato	Aug 2016	435 m	07º14'08' S	39º22'09" W	25.5 mm
E2	Pentecoste	Mar 2018	86 m	3º47' S	39º16'13" W	452.2 mm
E3	Crateús	Sep 2018	275 m	5°16'05" S	40°50'01" W	7.2 mm
E4	Madalena	Feb 2019	302 m	4º47'43" S	39º39'24" W	184.3 mm
E5	Bela Cruz	Jul 2019	9 m	3º04'48" S	40º06'37" W	3.1 mm
E6	Limoeiro do Norte	Jan 2020	143 m	5º10'59.4" S	38º00'21" W	426.7 mm

Source: adapted from Funceme (2021)

Grain brightness was analysed under storage in genotypes selected for adaptability and stability, using the excluded specular reflection method with a colorimeter (ColoQuest XE, HunterLab, United States). The moisture in the grains was standardised at 12% using the low-temperature oven method (Brazil, 2009) at the Seed Analysis Laboratory (LAS) of the Federal University of Ceará (UFC). The grains were packed and sealed in 20 µm polyethylene bags. Each package contained 500 g of beans and was stored under ambient conditions (25 ºC ± 5 ºC and 55% ± 15%). The following storage times were evaluated: Time I (harvest), Time II (2 months), Time III (4 months), Time IV (6 months) and Time V (8 months).

Data analysis

After verifying the normality of the data for grain productivity and homoscedasticity of the variances, individual and joint analyses of variance (ANOVA) were carried out. In the individual analyses, the adopted model was:

$$Y_{ij} = \mu + G_i + B_j + \varepsilon_{ij} \quad (1)$$

where: Y_{ij} is the phenotypic value of genotype i in block j; μ is the overall mean; G_i is the effect of the ith genotype; B_j is the effect of the jth block; ε_{ij} is the error associated with the ith genotype in the jth block.

In order to identify possible genotype x environment interactions, a joint analysis of variance was carried out as per the following model:

$$Y_{ijk} = \mu + G_i + A_j + Ga_{ij} + \varepsilon_{ijk} \quad (2)$$

where: Y_{ijk} is the phenotypic value of genotype I in environment j and block k; μ is the overall mean of the trait; Gi is the effect of the ith genotype, considered fixed; A_j is the effect of the jth environment, considered random; G_{Aij} is the effect of the interaction of genotype I with environment j, considered random; ε_{ijk} is the random error associated with the ith genotype in the jth environment and kth block.

Decomposition of the mean square error of the interaction into simple and complex parts was then estimated using the expression proposed by Cruz and Castoldi (1991). The mean values were then grouped using the Scott-Knott test at 5% probability. The analyses were carried out using the GENES software (Cruz, 2013).

The GGE-Biplot method was used to evaluate the adaptability and stability of the genotypes, separating the rainfed and irrigated environments. The GGE method considers two sources of variation (G + GE) without separating the effect of the genotype and of the interaction (Yan *et al*., 2007), as shown in the following equation:

$$Y_{ij} - \mu - B_j = Y_{i1}\alpha_{j1} + Y_{i2}\alpha_{j2} + \varepsilon_{ijk} \quad (3)$$

where: Y_{ijk} is the mean grain yield of genotype i in environment j; μ is the overall mean; B_j is the effect of environment j; γ_{i1} and α_{j1} are the main scores of genotype i and environment j, respectively; γ_{i2} and α_{j2} are the secondary scores of genotype i and environment j, respectively; ε_{ijk} is the residue not explained by any of the effects.

The GGE Biplot graphs were generated by the simple dispersion of γ_{i1} and γ_{i2} for the genotypes and α_{j1} and α_{j2} for the environments using singular value decomposition, as in the following equation:

$$Y_{ij} - \mu - B_j = \lambda_1\xi_{i1}\eta_{j1} + \lambda_2\xi_{i2}\eta_{j2} + \varepsilon_{ijk} \quad (4)$$

where: λ_1 and λ_2 are the largest eigenvalues for principal components 1 and 2 (PCA1 and PCA2), respectively; ξ_1 and ξ_2 are the eigenvalues of genotype i for PCA1 and PCA2, respectively; η_1 and η_2 are the eigenvalues of environment j for PCA1 and PCA2, respectively.

The accuracy was estimated as per Resende (2002):

$$A = \left(1 - \frac{1}{F}\right)^{\frac{1}{2}} \quad (5)$$

where: F is the value of the variance ratio for the effect of the genotypes associated with the ANOVA.

The environments were classified as favourable or unfavourable based on the Annicchiarico method

(1992), and a heatmap was generated to visualise the performance of the genotypes in the different environments. The analyses were carried out using the *metan* package (Olivoto; Lúcio, 2020) of the R software (R CORE TEAM, 2017).

The assumptions were met for the brightness data, and an analysis of variance and mean value test (Scott-Knott) were carried out for the six most adapted and/or stable genotypes. The ANOVA was carried out in a simple 6 x 5 factorial scheme (6 genotypes x 5 storage times).

RESULTS AND DISCUSSION

The joint analysis of variance showed a significant difference between the genotypes ($p < 0.05$) and environments ($p < 0.01$), as well as for the genotype x environment interaction ($p < 0.01$) (Table 3). This shows that the genotypes had different behaviours for grain yield, and that this variable was also influenced by the growth environments, in addition to there being an interaction between these factors. Similar results were obtained when evaluating the yield of cowpea genotypes in different environments in Brazil and in other areas of production (Abiriga *et al*., 2020; Araméndis-Tatis; Cardona-Ayala; Espitia-Camacho, 2021; Cruz *et al*., 2020; Melo *et al*., 2020; Sousa *et al*., 2017; Tomaz *et al*., 2022).

The significant G x E interaction explains the evaluations of adaptability and stability (Araméndiz-Tatis; Cardona-Ayala; Espitia-Camacho, 2021), especially when it comes to a complex trait such as grain yield (Abiriga *et al*., 2020). Furthermore, the predominance of the complex part of the G x E interaction confirms that the behaviour of the strains varied greatly in the environments under evaluation. However, the accuracy is considered high (Resende, 2002), denoting the high reliability of the recommendation process.

The genotype x environment interactions were complex for grain yield (Figure 2). A different genotype ranking can be seen in the environments under evaluation, which were classified as favourable or unfavourable for cultivation based on Annicchiarico (1992). This method helps to identify stable genotypes, which should have low sensitivity to unfavourable environments (Pereira *et al*., 2009). The environments Crato, Pentecoste, Crateús and Bela Cruz were classified as favourable, while Madalena and Limoeiro were unfavourable. In the trials, grain yield ranged from 279.4 to 2250.4 kg ha^{-1} for Genotypes 13 (BRS-Tumucumaque) and 4 (Bico-de-ouro 1-5-24), respectively.

Complex genotype x environment interactions are more challenging for breeders (Evangelista *et al*., 2021) as they make broader recommendations difficult. Due to these interactions, the analyses of adaptability and stability are even more important for selecting and recommending the evaluated strains more precisely (Cruz *et al*., 2021). The GGE Biplot method becomes highly relevant in these cases, as it affords greater precision when making the selection (Cruz *et al*., 2020).

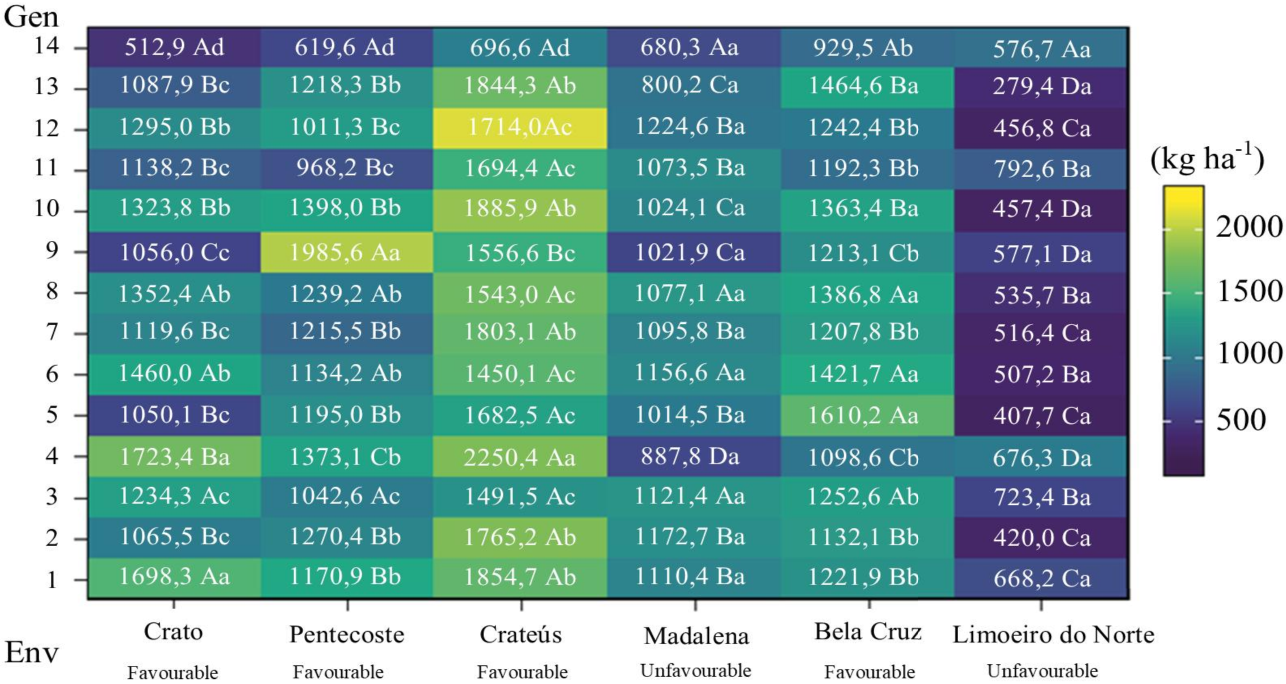
The two principal components generated by the GGE analyses (PC1 and PC2) explained 89.47% and 86.94% of the total variation in the grain yield data for the irrigated and rainfed environments, respectively (Figure 3). This shows that the biplots represented the existing interactions well, the components becoming even more representative as the cultivation systems separated. The first two components capture less information as the environments under evaluation increase (Tomaz *et al*., 2022).

Table 3 - Summary of the joint variance analysis, the simple and complex parts of the genotype x environment interaction, and accuracy for grain yield (kg ha^{-1}) in 14 cowpea genotypes evaluated in six locations in the state of Ceará

Source of Variation	Degrees of Freedom	Mean square
Genotypes (G)	13	561726.04*
Environments(E)	5	7436094.79**
G x E	45	263749.43**
Residual	164	92309.87
Part of the interaction G x E (%)		
Simple	-	26.39
Complex	-	73.61
Mean	-	1154.58
Accuracy	-	0.83

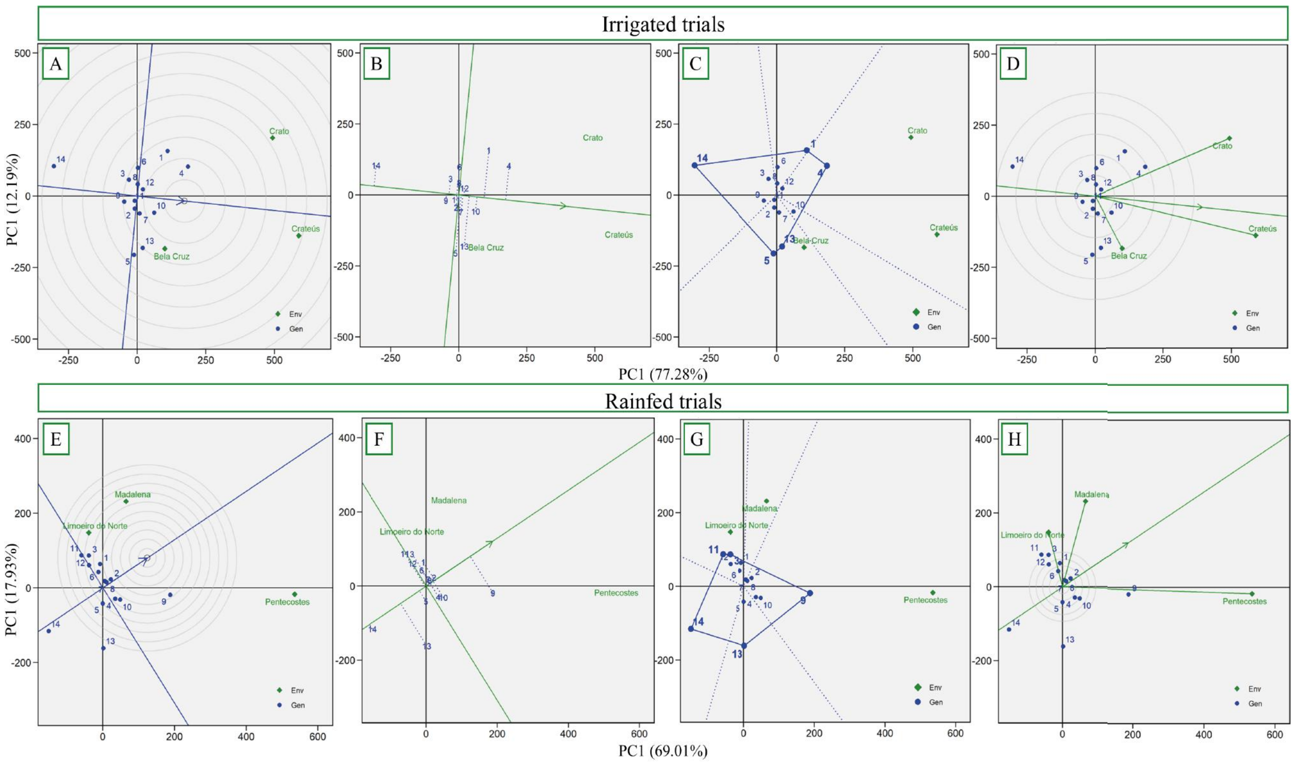
Source: prepared by the author

Figure 2 - Heatmap, grouping of average yield values (kg ha^{-1}), and the classification as per Annicchiarico (1992) for the different environments



Source: prepared by the author. Gen: genotypes. Mean values followed by the same uppercase letter on a line and/or the same lowercase letter in a column are part of the same grouping by Scott-Knott test at 5% probability

Figure 3 - GGE Biplot for the trials in the irrigated and rainfed environments



Source: prepared by the author. A and E: Genotype ranking; B and F: Mean vs. stability; C and G: Who wins where; D and H: Discrimination vs. representation. A, B, C and D: Irrigated trials. E, F, G and H: Rainfed trials

In the GGE Biplot (G + GE), the first principal component (PC1) correlates grain yield with the effect of the genotype, while the second component (PC2) summarises the sources of variation that lead to large differences in the yield of these genotypes between locations, representing the genotype x environment interaction (Cruz *et al*., 2020; Yan *et al*., 2000).

In Figure 3, graphs A and E show the ranking of the genotypes, where the arrow in the centre of the concentric circles marks the same distance between the origin and the longest vector of the environments, representing the ideal genotype. This genotype would have high grain yield in all the environments under evaluation (Melo *et al*., 2020; Yan; Tinker, 2006). As such, individuals located closest to this ideotype, such as Genotypes 4 and 10 for irrigated environments and Genotypes 9 and 2 for rainfed environments, had the highest grain productivity and stability in these locations. The different results for environments with different water regimes highlight the importance of separating the analyses of adaptability and stability for these production systems.

Graphs B and F (Figure 3) are entitled 'Mean vs. Stability' and show that genotypes with greater projection on the PC2 axis had lower stability albeit higher productivity in the environments closest to their position. The arrow indicates the coordinate of the average environment, which is the point of greatest stability (Cruz *et al*., 2020).

Genotype 10 was stable when irrigated, but did not have the highest average, while genotype 13 stood out in the Bela Cruzand Crateús environments (greater adaptability), but did not have the same performance in the Crato environment, showing less stability. Genotype 10 was also considered stable when evaluated in the state of Rio de Janeiro under different soil and climate conditions (Cruz *et al*., 2020).

Under rainfed conditions for example, Genotype 8 showed stable behaviour in each of the three environments, but also did not stand out in terms of productivity. Stable genotypes show similar behaviour over a range of environments, while adapted genotypes benefit under specific conditions. In each case, only those that meet the objectives of the program should be analysed and selected (Carvalho *et al*., 2016).

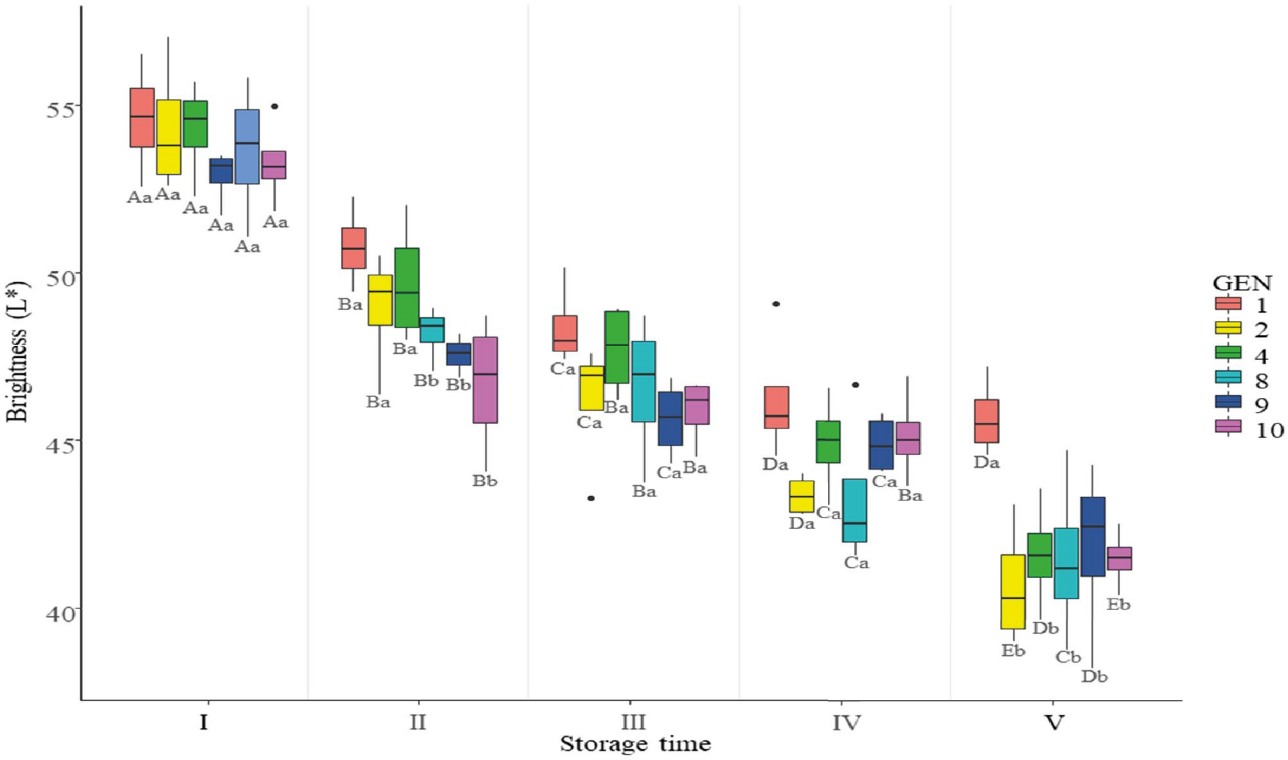
The GGE Biplot analysis is able to delimit mega environments in which the pattern of the genotype x environment interaction is similar, representing simple interactions or interactions with smaller changes in genotype ranking (Carvalho *et al*., 2016). Figure 3-C shows the formation of two mega environments, with Genotypes 4 and 13 located at the vertices, classifying them as more responsive to these locations. In Figure 3-G, Genotypes 9 and 3 were the most adapted to the three mega environments that were formed.

The GGE Biplot method is also able to classify the environments under analysis based on their representativeness and ability to discriminate between genotypes. The length of the environment vector in relation to PC1 is linked to its discriminating ability (Cruz *et al*., 2020; Yan *et al*., 2000; Yan; Tinker, 2006). Figures 3-D and 3-H show that Crateús (E3) and Pentecoste (E2) were therefore the most discriminating among those evaluated for irrigated and rainfed cultivation, respectively. These environments were also the most representative, due to the smaller angle formed with the axis of the average environment (Tomaz *et al*., 2022). Environments that form acute angles to each other show a positive correlation, while environments whose axes form obtuse angles are negatively correlated (Yan; Tinker, 2006). These results corroborate the classification made by Annicchiarico, since the correlation between favourable and unfavourable environments, such as E1 with E5 or E2 with E6, was small or non-existent. Melo *et al*. (2020) and Tomaz *et al*. (2022) also found positive and negative correlations between environments in cowpea cultivation in the state of Ceará when using the GGE Biplot method. It is, therefore, essential to identify genotypes for each condition due to the significant differences between the environments under evaluation. Furthermore, the design of genetic improvement programs for the cowpea must take this into account, developing strategies that enable efficient recommendations to be made.

There was a significant difference in grain darkening under storage between the six genotypes selected for their adaptability and stability (1, 2, 4, 8, 9 and 10) ($p < 0.01$). Storage times were also significant ($p < 0.01$), albeit with no interaction. There was a reduction in the brightness (L*) of all the genotypes over time; however, Genotype 1 showed less darkening compared to the other genotypes, with no difference in brightness between six and eight months of storage, and differing from the others statistically in the final evaluation (Figure 4). In the colorimetric analysis, brightness is the most important variable for detecting grain darkening, as it evaluates the lightness of the colour of the seed coat, ranging from black to white (Ribeiro; Jost; Cargnelutti Filho, 2004).

Genotypes 2, 4, 8, 9 and 10 showed a reduction of more than 21.5% in grain brightness with storage, while Genotype 1 showed a reduction of only 16.3%. The genotype that darkened the most after eight months of storage was Genotype 2, with a 25.8% reduction in brightness. Ribeiro, Jost and Cargnelutti Filho (2004) give an ideal brightness of greater than 53 for carioca beans, and state that this value should not change with time or the environmental conditions; however, they highlight the possibility of this change. The brightness of the genotypes selected here ranged from 52.9 to 54.6 at the time of harvest (Time I); by the end of the eight months of storage, the brightness ranged from 41.5 to 45.7.

Figure 4 - Boxplot of grain brightness (L*) in selected cowpea genotypes under storage



Source: prepared by the author. Time I: harvest; Time II: 2 months; Time 3: 4 months; Time 4: 6 months; Time 5: 8 months

Genotype 1 had the highest average in the Crato environment and the third highest average in Crateús, both under irrigated conditions. It was unstable in each of the environments under evaluation, but can be recommended as an alternative cultivar with less grain darkening in the locations to which it adapted best. Genotype 9, considered stable, had a higher grain yield than the controls in each of the evaluated environments, and was second only to Genotype 1 in terms of less darkening. Cruz *et al*. (2020) also recommended the genotype for its stability.

For these cultivars to become available on the market, it is important they be launched and publicised, and the farmers and seed producers encouraged. In the seed distribution program for farmers in the state of Ceará, only two cultivars (Pujante and IPA 207 Miranda) were available last year due to a lack of offers from the bidders (Ceará, 2021). The distribution of seeds from stable or adapted cultivars in certain locations in the state can help increase the average productivity of the cowpea in Ceará, of 329 kg ha^{-1} (EMBRAPA, 2020).

CONCLUSIONS

1. Genotype 9 can be recommended for the semi-arid region of the state of Ceará as it is stable and has good grain yield. Genotypes 1, 4, 8 and 10 are the most adapted to irrigated environments, and Genotypes 1, 2, 7 and 9 to locations with rainfed cultivation;
2. Genotype 1, in addition to its good productive performance, shows less grain darkening under storage, and can be recommended to producers and resellers who stock the product, so as to guarantee better post-harvest quality.

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