

Selection of superior cowpea lines for multi-traits and adaptabilities to the Piauí semi-arid using genotype by yield*trait biplot analysis

Seleção de linhagens de feijão-caupi superiores para múltiplos caracteres e adaptações ao semiárido piauiense por meio da Análise genotype by yield*trait biplot

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Received in June 5, 2021 and approved in August 20, 2021

ABSTRACT

Cowpea is a great socioeconomically important leguminous plant worldwide. The crop is an excellent source of proteins and minerals, mainly for the poorest populations. It is cultivated in some states of Brazil. The Piauí semi-arid is the most yielding zone in the state. Thus, the selection of superior cowpea genotypes for multi-traits and adaptabilities to this growing area is very important for the small-scale and medium-scale farmers in Piauí. The objective of this study was to select the superior cowpea lines for their multi-traits and adaptabilities to the Piauí semi-arid zone based on the genotype by the yield*trait (GYT) Biplot approach. Seventeen elite lines and three cultivars were evaluated in a randomized complete block design with four replications under the rainfed growing condition at three different locations away from the Piauí semi-arid zone. The following traits were evaluated: the number of days to flowering, pod length, the number of grains per pod, weight of one hundred grains, grain index, grain yield, Fe and Zn contents, and also the cooking quality. The analysis of variance (ANOVA), the grouping of means, and simultaneous selection by the GYT Biplot were implemented. Through the GYT Biplot approach, the two lines, MNC11-1013E-35 and MNC11-1013E-15, were found superior because they combined the traits, grain-yield with the yield components and nutritional traits simultaneously during the cultivation in the Piauí semi-arid zone. However, the MNC11-1052E-3 line was better in the combination of yield and cooking quality than the other lines.

Index terms: *Vigna unguiculata*; GYT Biplot; nutri-culinary values.

RESUMO

O feijão-caupi é uma leguminosa de grande importância socioeconômica no mundo. A cultura constitui uma excelente fonte de proteínas e minerais, principalmente para a população mais pobre. No Brasil, é cultivada em alguns estados. O semiárido piauiense é a zona de maior produção do Estado. Assim, a seleção de genótipos superiores de feijão-caupi para múltiplos caracteres e adaptados a esta área de cultivo é importante para os pequenos e médios agricultores do Piauí. O objetivo deste estudo foi selecionar linhagens superiores de feijão-caupi para múltiplos caracteres e adaptados à zona semiárida do Piauí, com base na abordagem *Genotype by Yield*Trait* (GYT) Biplot. Dezesete linhagens elite e três cultivares foram avaliadas em delineamento de blocos casualizados com quatro repetições, em regime de sequeiro em três ambientes do semiárido piauiense. Os seguintes caracteres foram avaliados: número de dias para início do florescimento, comprimento da vagem, número de grãos por vagem, peso de cem grãos, índice de grãos, produtividade de grãos, teores de Fe e Zn, e qualidade de cozimento. Análise de variância, agrupamento de médias e seleção simultânea por GYT Biplot foram implementados. Por meio da abordagem GYT Biplot, as linhagens MNC11-1013E-35 e MNC11-1013E-15 foram superiores porque combinaram a produtividade de grãos simultaneamente com os componentes de produção e nutricionais no cultivo do semiárido piauiense, porém, MNC11-1052E-3 foi melhor na combinação de produtividade e qualidade de cozimento do que as outras linhagens.

Termos para indexação: *Vigna unguiculata*; GYT Biplot; valores nutricionais e culinários.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is originally from the African continent (Xiong et al., 2016; Karapanos et al., 2017). The cultivation occurs mainly in Africa, Southeastern Asia, Southern United States, and Latin America (Xiong et al., 2018; Aliyu et al., 2019; Baoua et al., 2021). However, the crop has great socio-economic and nutritional importance, especially for the poorest population (Gondwe, 2019). Brazil is the third world cowpea producing country with the harvested area, production, and yield of 1,309.00 ha⁻¹, 687.40 tonnes, and 525 kg ha⁻¹, respectively, in the 2020/2021 agricultural year. The Northeastern region accounts for most of the national production (64.15%), followed by the Midwestern (22.29%) and Northern (10.87%) regions (Companhia Nacional de Abastecimento - CONAB, 2021).

Cowpea has a short maturation cycle with satisfactory adaptability to the conditions like drought (Spriggs et al., 2018; Carvalho et al., 2019), low water requirement, and also little need for manures and fertilizers for cultivation (Boukar et al., 2018). Cowpea is able to fix biological nitrogen with the help of nitrogen-fixing microorganisms, such as *Rhizobia*, contributing to the soil quality (Ddamulira et al., 2015; Mohammed et al., 2018). The crop is ideal for genetic breeding studies and biofortification as it is mainly a strategic food for fighting against human malnutrition and diseases (Dakora; Belane, 2019; Coelho et al., 2021).

More than two billion people worldwide suffer from micronutrient and vitamin deficiencies (World Health Organization - WHO, 2019). The target populations were children under the age of five years and women of reproductive age (Bailey et al., 2015). One way to mitigate this problem is ingesting bio-fortified food, considering it a promising strategy for the poorest populations (Saeid et al., 2019). Cowpea is mainly known for its nutritional richness, especially for the high protein content (20%-30%) (Boukar et al., 2016; Gerrano et al., 2019a) as well as starch, fibers (Akuru et al., 2021), pro-vitamin A, iron (Fe), zinc (Zn), phosphorus (P), and carotenoids, such as lutein, β -carotene, γ -carotene, and cryptoxanthin (Ojwang et al., 2013; Alidu et al., 2020). Embrapa Meio-Norte, located in the state of Piauí, has been developing bio-fortified cultivars intending to combine the high yielding, nutritional, and culinary quality traits simultaneously (Empresa Brasileira de Pesquisa Agropecuária - Embrapa, 2010).

Multiple trait selection is a promising breeding strategy for selecting the superior genotypes, which has been carried out through some methodologies, such as the tandem method, independent levels of selection, and

selection index (Burdon; Klápště, 2019). However, the studies addressing the selection of cowpea genotypes based on multi-traits in semi-arid conditions (environments with low fertility soils, low index, and irregularity of rainfall and high temperature) are rare. In addition, the breeding has found it difficult to gather the high yielding, excellently nutritive, and high cooking quality traits in a single genotype due to the genotype-by-environment interaction (GEI) and an unfavorable association between the desired traits (Gerrano et al., 2020).

The factors considered above are of paramount importance when selecting a cultivar with broad or specific adaptability for its given growing region. Yan and Frégeau-Reid (2018) developed the "Genotype by Yield*Trait" (GYT) Biplot approach to select the superior genotypes based on the multi-traits. Considering the socio-economic importance of cowpea plants in certain regions of the world (Kebede; Bekeko, 2020), and mainly in Northeastern Brazil, that approach allows combining the yield with other key traits of the breeding lines (Yan; Frégeau, 2018; Yan et al., 2019). The selection of multi-traits has been carried out in the cowpea breeding and, most commonly, through the selection indexes (Torres et al., 2016; Omirou et al., 2019) with few studies using the GYT Biplot approach (Oliveira et al., 2019; Cruz et al., 2020; Araújo et al., 2021). According to the latter authors, the methodology allows the selection of superior cowpea lines by focusing on the combinations between yield and other traits of interest with easily interpreted results. The objective of this work was to select superior cowpea lines for multi-traits in the Piauí semi-arid zone of northeastern Brazil, following the GYT Biplot approach.

MATERIAL AND METHODS

Planting materials

Twenty cowpea genotypes were evaluated, comprising 17 elite lines and three commercial cultivars (Table 1).

The cultivar BRS Pajeú had the traits like mulatto grains, Fe and Zn richness, and fast cooking. The cultivation was aimed at the family and business farmers standing out mainly in the Caatinga, Cerrado, and Tabuleiros Costeiros ecosystems under rainfed or irrigated regimes of the northeastern region (Embrapa, 2009). The cultivar BRS Marataoã had a plant architecture suitable for mechanical harvesting and desiccation. The cultivar was of the evergreen subclass, which was recommended for rainfed cultivation in the states of Piauí, Paraíba, and Bahia (Freire Filho et al., 2004). The BRS Rouxinol cultivar was selected for cultivation under the rainfed and irrigated conditions by

the family and business farmers in the state of Bahia with an average yield of 1,500 kg ha⁻¹ (Embrapa, 2002).

These genotypes correspond to the treatments of the Cultivation and Use Value trials of the Cowpea Breeding Program from Embrapa Meio-Norte, in Teresina, PI, Brazil. These trials are necessary for the registration of new cultivars by the National Cultivar Registry of the Ministry of Agriculture, Livestock, and Supply from Brazil (Ministério da Agricultura, Pecuária e Abastecimento - Mapa, 2019).

Environments for trials

The field experiments were performed in three different municipalities within the Piauí semi-arid region, in the State of Piauí, Brazil: Monsenhor Hipólito (MSH) (07°00'06" S, 41°01'46" W, the altitude of 262 m, and the mean annual rainfall between 800 mm and 1400 mm), Pio IX (PIX) (06°50'15" S, 40°34'45" W, the altitude of

494 m, and the mean annual rainfall of 700 mm), and São Miguel do Tapuio (SMT) (05°29'46" S, 41°18'46" W, the altitude of 272 m, and the mean annual rainfall between 800 mm and 1400 mm).

The experiments were in the randomized block design with four replications. The treatments were represented by a plot of four 5.0 m rows with the plants spacing of 0.50 m 0.25 m for each, totaling a useful area of 5 m² shown by the two central rows for data collection. The soil samples of the three sites were Red-Yellow Podzolic type. However, the minimum as well as maximum temperatures, and the annual precipitation were 25 °C, 38°C, and 1,100 mm in MSH; 18 °C, 36 °C, and 700 mm in PIX, whereas 20 °C, 32 °C, and 700 mm in SMT. The trials were carried out under rainfed growing conditions, where the climate was tropical, and the weather was dry wintry (Kottek et al., 2006; Alvares et al., 2013).

Table 1: The genealogy of twenty different cowpea genotypes from the Embrapa Meio-Norte breeding program, Teresina, Brazil.

Code	Line	Genealogy	Commercial subclass
G1	MNC11-1013E-33	MNC01-510F x Pingo-de-Ouro-1-2	ML ¹
G2	MNC11-1013E-16	MNC01-510F x Pingo-de-Ouro-1-2	EG
G3	MNC11-1013E-15	MNC01-510F x Pingo-de-Ouro-1-2	ML
G4	MNC11-1013E-35	MNC01-510F x Pingo-de-Ouro-1-2	EG
G5	MNC11-1018E-17	MNC02677F-2-2 x MNC01-631-20-5 x Pingo de Ouro-1-2	EG
G6	MNC11-1019E-8	MNC01-631F-11 x Canapuzinho-2 x MNC02-677F-2-1	ML
G7	MNC11-1019E-12	MNC01-631F-11 x Canapuzinho-2 x MNC02-677F-2-1	ML
G8	MNC11-1019E-46	MNC01-631F-11 x Canapuzinho-2 x MNC02-677F-2-1	ML
G9	MNC11-1020E-16	MNC02-689F-11 x MNC02-677F-2-1	ML
G10	MNC11-1022E-58	MNC02-689F-11 x MNC01-631F-20-5 x MNC99-510F-16-1	ML
G11	MNC11-1024E-1	MNC02-689F-11 x MNC99-510F-16-1 x Pingo-de-Ouro-1-2	ML
G12	MNC11-1026E-15	MNC02-689F-11 x MNC01-631F-11 x Canapuzinho-2	ML
G13	MNC11-1026E-19	MNC02-689F-11 x MNC01-631F-11 x Canapuzinho-2	ML
G14	MNC11-1031E-5	MNC02-689F-11 x MNC02-680F-1-2	EG
G15	MNC11-1031E-11	MNC02-689F-11 x MNC02-680F-1-2	ML
G16	MNC11-1034E-2	MNC01-631F-20-5 x Pingo-de-Ouro-1-2 x MNC02-761F-2	ML
G17	MNC11-1052E-3	Bico de Ouro-1-2-1 x MNC01-631F-20-5 x MNC99-510F-16-1	CN
G18	BRS Pajeú	CNCx405-17F x TE94-268-3D	ML
G19	BRS Marataoã	Seridó x TVx1836-013J	ML
G20	BRS Rouxinol	TE86-75-57E x TEx1-69E	EG

¹ML-Mulate; EG-Evergreen; CN-Canapu.

Measurement of traits

The following traits were evaluated: NDF = number of days to flowering (the number of days after planting, where 50% of the plants in a useful area had flowers); PL = pod length (average length measured using five pods taken at random in the useful area of the plot); NGP = number of grains per pod (the average number of grains obtained from five pods taken at random in the plot); WHG = weight of one hundred grains (the total weight of one hundred grains taken at random); GI = grain index (ratio of the weight of grains to the weight of pods obtained from five pods taken at random in the experimental area); YLD = grain yield (ratio of the weight of grains to the weight of pods obtained from five pods taken at random in the experimental unit); Fe = iron content; Zn = zinc content; PC = protein content, and CQ = cooking quality.

Nutritional analysis

Nutritional analysis was carried out in the Bromatology laboratory of Embrapa Meio-Norte, Teresina, Piauí, Brazil. The grain samples of 200 g of each genotype per location were used. Initially, the grains were washed with the distilled water and later kept in paper bags to place in an oven at 60 °C for 48 h. After drying, the grains were crushed in a zirconium ball mill. Then the analyses of Fe, Zn, and protein contents were performed with the flour obtained from the crushed grains.

Determination of Fe and Zn contents

The Fe and Zn contents in the grains of the genotypes were determined according to the methodology of Sarruge and Haag (1974). The sample flour (200 mg per tube) was transferred to the digestion tubes, and 5 mL of digestion solution (nitro-perchloric solution, 2:1) was then added to each of the tubes. Next, the tubes were placed in the block digester for approximately two hours until 200 °C was reached. The extracts of approximately 2 mL appeared transparent and clear after the digestion. Then, distilled water was added to make up the volume of 20 mL, and the tubes were shaken in a vortex mixer. The flame atomic absorption spectroscopy of the prepared solutions was performed (iCE 3000, USA). The Fe and Zn concentrations were obtained in the parts per million (ppm) unit and then converted to the mg kg⁻¹ unit. All analyses were performed in triplicate.

Determination of protein content

According to the methodology of Aoac International (2005), the amount of protein content was determined by

the Micro-Kjeldahl method using a conversion factor of 6.25. The sample flour of 200 mg was weighed and homogenized on parchment paper and then placed in the Kjeldahl protein digestion tube. Along with the sample, 2 g catalytic mixture (96.5% potassium sulfate and 3.5% copper sulfate) and 5 mL sulfuric acid were measured and mixed. The sample was digested in a digester at 420 °C temperature for 1 h 40 min. Soon after, 10 mL distilled water was added to start the distillation in a nitrogen/protein distiller. A total of 15 mL of 50% sodium hydroxide solution was added to the tube to boil, and then the ammonia present in the sample was dragged by the steam to an Erlenmeyer with boric acid and red methyl indicator.

The amount of nitrogen was determined by means of titration with 0.02 N hydrochloric acid solution, and the protein content (%) was obtained from Equation 1:

$$\text{Total nitrogen} = (\text{VHA} \times \text{F} \times 0.14) / \text{P} \quad (1)$$

where:

VHA = the volume of hydrochloric acid used in titration;
F = the correction factor for the hydrochloric acid solution;
P = weight of the sample in grams.

The amount of protein content (%) was calculated using Equation 2:

$$\text{Protein content (\%)} = \text{Total nitrogen} \times 6.25 \quad (2)$$

Determination of cooking quality

The cooking quality evaluation followed the methodology of Carvalho et al. (2017) with an adaptation in the soaking time. Samples of 25 grains from each genotype without any mechanical damage were placed in the voile bags. Two bags per genotype were used corresponding to the two replications/genotypes/locations. Bags containing the samples were placed in distilled water for 60 min. An electric pressure cooker (5 L) with a water level of three-fifths of the total pan volume was used. The samples were cooked for 30 min and then placed on a counter for cooling at room temperature for five minutes.

The evaluation of the percentage of cooked grains was carried out with the help of a Mattson cooker, where 25 grains per sample were chosen at random and used. Next, the pins were placed in the grains, and the number of pins able to completely pierce them was recorded. The sample was considered as cooked when 13 of the 25 grains (around 52%) were completely perforated (Mattson, 1948).

Statistical analysis

Individual and joint analyses of variance were performed, and the means were grouped by the Scott-Knott test (p -value < 0.05) for all the traits. The effect fixed for a genotype and random environment was considered on analysis. The individual variance analysis of the agronomic traits as well as cooking quality was performed according to Equation 3.

$$Y_{ij} = \mu + G_i + B_j + \varepsilon_{ij} \quad (3)$$

where:

Y_{ij} : the observation of the genotype i on block j ;
 μ : overall;
 G_i : the effect of the genotype i ;
 B_j : the effect of the block j ;
 ε_{ij} : the effect of the error associated with the observation ij .

The statistical model adopted for analyzing the joint variance of the agronomic traits and cooking quality was according to Equation 4.

$$Y_{ijk} = \mu + (B/E)_{jk} + E_j + G_i + GE_{ij} + \varepsilon_{ijk} \quad (4)$$

Y_{ijk} : the observation of the genotype i in the environment j and block k ;
 μ : overall;
 B/E_{jk} : the effect of the interaction of block k within the environment j ;
 E_j : the effect of the environment j ;
 G_i : the effect of the genotype i ;
 GE_{ij} : the effect of the interaction between the genotype i and environment j ;
 ε_{ijk} : the error associated with the observation ijk .

The completely randomized design was used for analyzing the Fe, Zn, and protein contents. The individual analysis of variance was made according to Equation 5:

$$Y_{ij} = \mu + G_i + \varepsilon_{ij} \quad (5)$$

where:

Y_{ij} : the observation of the genotype i in the replication j ;
 μ : overall;
 G_i : the effect of the genotype i ;
 ε_{ij} : the error associated with the observation ij .

The statistical model adopted for the analysis of joint variance of Fe, Zn, and protein contents were according to Equation 6:

$$Y_{ij} = \mu + G_i + E_j + GE_{ij} + \varepsilon_{ij} \quad (6)$$

where:

Y_{ij} : the observation of the genotype i in the environment j
 μ : overall;
 G_i : the effect of the genotype i ;
 E_j : the effect of the environment j ;
 GE_{ij} : the effect of the interaction between genotype i and the environment j ;
 ε_{ij} : the error associated with the observation ij .

The simultaneous selection was made using the Genotype by Yield*Trait (GYT) Biplot approach (Yan; Fréreau-Reid, 2018). This analysis was based on the information from phenotypic means. When the breeder intended to increase the trait, this value, for example, the weight of one hundred grains (YLD*WHG), was multiplied by the grain yield value. On the other hand, when the breeder's interest would be to reduce the trait, the average of the trait, for example, the number of days to flowering (YLD/NDF), would be divided.

The data for GYT analysis was standardized so that the mean of each trait or yield-trait combination became zero and the variance became a unit. The standardization was performed following Equation 7:

$$P_{ij} = (T_{ij} - \bar{T} / S_j) \quad (7)$$

where:

P_{ij} : the standardized value of the genotype i for the trait or grain yield-trait combination j on the standardization table;
 T_{ij} : the original value of the genotype i for the trait or grain yield-trait combination j on the GT or GYT table;
 \bar{T} : the mean across the genotypes for the trait or grain yield-trait combination j ;
 S_j : the standard deviation for the trait or grain yield-trait combination j .

The GYT Biplot was based on the first two principal components (PC) obtained from the singular value decomposition (SVD). However, the standardization was performed for all data used in the simultaneous selection. The SVD of the GYT table was converted to genotype eigenvalues, trait eigenvalues, and singular values according to the Equation 8 proposed by Yan and Fréreau-Reid (2018):

$$P_{ij} = (d\lambda_1^\alpha \xi_{i1}) \left(\frac{\lambda_1^{1-\alpha} \tau_{1j}}{d} \right) + (d\lambda_2^\alpha \xi_{i2}) \left(\frac{\lambda_2^{1-\alpha} \tau_{2j}}{d} \right) + \varepsilon_{ij} \quad (8)$$

where:

ξ_{i1} e ξ_{i2} = the Eigenvalues of PC1 and PC2, respectively, for the genotype i ;

τ_{1j} e τ_{2j} = the Eigenvalues of PC1 and PC2, respectively, for the grain yield-trait combination j ; λ_1 e λ_2 = Singular values of PC1 and PC2, respectively;

α = the singular factor of value partitioning;

d = the scalar distance that was chosen so that the longest vector length between the genotypes remained equal to the length between the traits;

ε_{ij} = the residual from fitting the PC1 and PC2 for the genotype i on the grain yield-trait combination j .

The analyses of individual and joint variances along with the grouping of averages were performed using the GENES program (Cruz, 2016). The GYT Biplot analysis was performed using the R statistical environment (R Core Team, 2020) with an aid of the GGEBiplotGUI package (Bernal; Villardon, 2020).

RESULTS AND DISCUSSION

Agronomical traits

The ANOVA showed that the genotypic effect was significant ($p < 0.05$) for the NDF, PL, NGP, WHG, and GI. Moreover, the effect of the environmental mean square was significant ($p < 0.01$) for all the traits. On the other hand, the G x E interaction was not significant (Table 2).

The existence of genetic variability was conducive in obtaining superior genotypes (Singh et al., 2020). Increasing grain yield has been one of the targets for cowpea breeding (Freire Filho et al., 2012; Rocha et al., 2017; Boukar et al., 2018). The lack of variation in the YLD trait had already been reported in the previous studies carried out under rainfed conditions in the Brazilian semi-arid zone (Rodrigues et al., 2017). This could be explained by the successive selection cycles carried out in previous generations to obtain high-yielding genotypes and make the genetic base narrow (Cobb et al., 2019). Additionally, many lines had common parents in their genealogies, which favored inbreeding.

The significant effect of the environment indicated its differentiated behavior towards the evaluation of the superior genotypes showing contrasts among them. A similar study evaluating the cowpea genotypes for selecting the promising parents to form an improved population showed the effect of significant environments, except the grain yield (Gerrano et al., 2019b). The genotypes for the evaluated agronomic traits behaved similarly in the testing environment due to the lack of significant differences between the G x E interaction effects and the genetic relatedness between the testing genotypes. However, the absence of G x E interaction was observed during the evaluation of agronomic performances, except for the plant format and grain yield of 16 cowpea lines in the semi-arid zone of Southeastern Brazil (Silva et al., 2018).

Table 2: Summary of ANOVA for the agronomic traits of cowpea in three environments of the Piauí semi-arid zone of Northeastern Brazil.

Source of variation	DF	Mean squares					
		NDF (day)	PL (cm)	NGP (unity)	WHG (g)	GI (%)	YLD (kg ha ⁻¹)
Block/Environment	9	15.96	0.52	0.08	5.13	7.37	277668.48
Environment (E)	2	398.08**	2.70**	5.15**	310.67**	174.01**	5063382.32**
Genotype (G)	19	38.21**	3.11**	0.88*	20.64**	65.10**	145237.51 ^{ns}
G x E	38	14.51 ^{ns}	0.55 ^{ns}	0.47 ^{ns}	1.86 ^{ns}	9.56 ^{ns}	89398.59 ^{ns}
Error	171	13.04	0.50	0.36	1.49	12.77	92861.26
Total	239						
Overall mean		49.71	11.11	7.78	18.51	75.57	1209.13
CV (%)		7.31	6.48	7.70	6.83	4.72	25.44

* $p < 0.05$, ** $p < 0.01$ (Snedecor's F-test). DF = degree of freedom. CV = coefficient of variation. G x E = genotype by environmental interaction; NDF = number of days to flowering; PL = pod length; NGP = number of grains per pod; WHG = weight of one hundred grains; GI = grain index; YLD = grain yield.

The mean values of the 20 test cowpea genotypes evaluated for the agronomic traits are presented in Table 3. Significant differences between the genotypes ($p < 0.05$) were identified for most of the agronomic traits, except the YLD.

The genotypes formed two groups for the NDF trait, where group “A” was composed of 55% of the evaluated genotypes. The overall mean of the experiment was 49.71, with the flowering time varying from 45 days to 52 days (Table 3). The selection of superior genotypes for a set of traits of economic interest is desirable in genetic breeding. Obtaining early materials is one of the promising strategies, as it can minimize crop loss in the summer or stabilize production in regions with prolonged periods of drought, protecting the plant from biotic or abiotic stresses Elteib et al. (2021), e.g., as the Brazilian semi-arid zone.

Similar values of the overall mean for NDF were obtained during the evaluation of cowpea genotypes in terms of earliness (Santos et al., 2014). Thus, the NDF

trait was desired since it could reduce the crop’s exposure to pests, especially in the semi-arid zones (Ehlers; Hall, 1997), improve water usage in the semi-arid areas, promote better adaptation to the “second crop” production system at the end of the water season (low amount of rain), and lower the production cost as well as the number of inputs due to the short growth period.

The PL trait was divided into three groups, and group “A” consisted of 70% of the genotypes ranging from 10.08 cm to 11.52 cm. The lengths of G20, G17, G9, and G20 were 10.32 cm, 10.46 cm, 10.54 cm, and 10.69 cm, respectively. The group “C” was made up of G18 and G19, which showed shorter pod lengths (Table 3). The overall mean of pod length of the Brazilian cowpea cultivars obtained during the period ranging from 2000 to 2015 was 18 cm. In the present study, 59% of the evaluated cowpea lines showed averages greater than 18 cm showing a considerable genetic gain with the selection made in the previous cycles (Freire Filho, 2012).

Table 3: Mean values of agronomic traits of the test cowpea genotypes evaluated in three environments in the Piauí semi-arid zone of Northeastern Brazil.

Genotype	NDF (day)	PL (cm)	NGP (unity)	WHG (g)	GI (%)	YLD (kg ha ⁻¹)
G1	48.25 b	10.93 a	7.62 b	17.25 c	75.54 b	1230.85 a
G2	46.25 b	10.69 b	7.03 b	17.24 c	75.60 b	1174.79 a
G3	51.58 a	11.28 a	7.61 b	19.54 a	79.03 a	1353.94 a
G4	50.58 a	11.13 a	8.06 a	18.76 b	77.40 a	1398.51 a
G5	52.25 a	11.52 a	8.19 a	18.47 b	74.49 b	1095.37 a
G6	49.67 a	11.52 a	7.64 b	18.74 b	74.06 b	1244.80 a
G7	51.83 a	11.08 a	7.92 a	19.14 a	79.80 a	1235.42 a
G8	45.58 b	11.02 a	7.84 a	18.46 b	75.04 b	1218.69 a
G9	50.92 a	10.54 b	7.72 b	15.79 d	77.52 a	1142.44 a
G10	49.75 a	11.24 a	7.98 a	17.96 b	73.68 b	1149.71 a
G11	49.17 b	10.08 a	7.65 b	17.88 b	76.71 a	1271.86 a
G12	50.08 a	11.15 a	7.68 b	18.55 b	76.71 a	1149.61 a
G13	48.17 b	11.30 a	8.03 a	16.82 c	74.75 b	1210.37 a
G14	50.00 a	11.36 a	7.77 b	19.41 a	77.25 a	1141.44 a
G15	47.25 b	10.95 a	7.54 b	17.42 c	76.93 a	1230.92 a
G16	50.67 a	11.51 a	7.77 b	19.32 a	74.03 b	882.73 a
G17	48.08 b	10.46 b	7.95 a	19.14 a	78.67 a	1339.51 a
G18	50.25 a	9.86 c	8.15 a	16.14 d	72.36 b	1186.38 a
G19	48.92 b	9.70 c	8.15 a	15.15 d	72.59 b	1207.88 a
G20	48.58 b	10.32 b	7.78 b	16.23 d	71.68 b	1088.62 a
Overall mean	49.71	11.11	7.78	18.51	75.57	1209.13

Different letters in the column, for the same trait, indicate significant differences at $p < 0.05$ (Scott-Knott’s test); NDF = number of days to flowering; PL = pod length; NGP = number of grains per pod; WHG = weight of one hundred grains; GI = grain index; YLD = grain yield.

The overall mean for the NGP was 7.78 grains. The values of genotypes ranged from 7.03 grains (G2) to 8.19 grains (G5) and were separated into two groups: the group “A”, which comprised 45% of the genotypes, and group “B” that gathered the genotypes with performances of nearly seven grains per pod (Table 3). The overall mean for pod length and the number of grains per pod observed in this study were below the Brazilian national mean for the crop, which corresponded to 20 cm of pod length and 13 grains per pod (Santos et al., 2014; Oliveira et al., 2015).

The ideotype with the highest number of pods per plant tends to have a lower number of grains per pod, which is desired by business farmers, as they demand upright cultivars and smaller pods to facilitate mechanized harvesting. However, small level farmers, who carry out manual harvesting, prefer cultivars with longer pods and a high number of grains per pod (Freire Filho et al., 2012).

The genotypes formed four groups for the WHG trait, with the variation ranging from 15.15 g (G19) to 19.54 g (G3). The group “A” gathered five lines (G3, G7, G14, G16, and G17) with a mean of 19.31 g, which was higher than the overall mean and the control mean. The lines proved to be superior for the WHG since they obtained a mean of 18.74 g, which was higher than the control value (16.14 g) and equal to the average mean (18.51 g). The groups B, C, and D had performances of 18.40 g, 17.18 g, and 15.83 g, respectively, which were below the overall mean. Similar values were obtained by Silva and Neves (2011) in an evaluation of 20 cowpea genotypes under rainfed conditions in the Brazilian Northeast region. The WHG had a direct correlation with the grain size. Generally, the Brazilian cowpea consumers prefer larger grains; therefore, the cowpea breeding program has been focused on selecting the grain ideotype by combining the color, shape, and the aspect of the tegument traits as per the consumers’ demand.

The overall grain yield was 1,209.13 kg ha⁻¹ (Table 3), which was higher than the mean (695 kg ha⁻¹) of the Brazilian national crop for 2018–2019 agricultural year (Conab, 2019), and these results were found by Rocha et al. (2017) for the cowpea genotypes responsible for grain yield trait evaluated in the Northeastern region of Brazil. Selecting cowpea genotypes with high grain yields was the main objective of the breeding programs. Although the genotypes have not shown any variability, the mean of the lines was considered high because of the several previous selection cycles for this trait exceeding the mean grain yield of Brazilian cowpea cultivars released throughout the last 20 years.

The GI ranged from 71.68 (G20) to 79.80 (G7). The lines were divided into two distinct groups with a higher average value than the overall and the control means. The variation in the grain yield trait showed a range from 882.73 kg ha⁻¹ (G16) to 1,398.51 kg ha⁻¹ (G4), with an overall performance of 1,209.13 kg ha⁻¹. However, for YLD, there was no significant difference between the lines. Similar grain index values were observed by Silva and Neves (2011) in the evaluation of 20 cowpea genotypes under rainfed conditions in the Brazilian Northeast.

The GI is a trait that represents the grain/shell ratio of the pod. Genetic improvement seeks a high grain index, which means more grains than bark in the pod; however, this index cannot reach 100%, as the pod would become very fragile and would lose much of the grain protection against the environment, in addition to facilitating dehiscence still in the field. An ideal grain index range is between 70% and 90%. The results observed in this work show that all lines are within this range, showing that they are close to the ideotype for that trait.

Nutritional traits

The effects of the genotypes were significant ($p < 0.01$) for the PC and CQ traits. The effects of environment and G x E interaction were significant for all the traits. The overall mean for the Fe, Zn, and protein contents were 51.10 mg kg⁻¹, 46.87 mg kg⁻¹, and 24.37%, respectively (Table 4). The results showed that the evaluated genotypes had genetic variability for the PC trait of cowpea grain and indicated that it was possible to obtain better selectively. On the other hand, significant differences between the effects of environments as well as the G x E interactions suggested that the test environments were contrasting for the nutrients and showed differences in behaviors between the genotypes with the environmental variations.

The results agreed with the statement by Jayathilake et al. (2018) that mentioned the wide genetic variability of minerals present in grains of the Brazilian genotypes that had high contents of those, such as 60 mg kg⁻¹ of Fe, 40 mg kg⁻¹ of Zn, and 30% of protein, respectively (Cruz et al., 2014). Furthermore, the protein content of cowpea was two to four times higher than that of other cereals and tubers (Sebetha et al., 2014), which might be related to the efficiency of some genotypes in assimilating nitrogen through its association with the bacteria of genus *Rhizobium* as they were responsible for carrying out the biological nitrogen fixation in the soil (Gomes et al., 2017).

Table 4: Summary of the ANOVA for nutritional traits in cowpea evaluated in three environments of the Piauí semi-arid zone of Northeastern Brazil.

Source of variation	DF	Mean squares		
		Fe (mg kg ⁻¹)	Zn (mg kg ⁻¹)	PC (%)
Genotype (G)	19	350.17 ^{ns}	41.66 ^{ns}	7.03 ^{**}
Environment (E)	2	1205.55 ^{**}	305.45 ^{**}	16.32 ^{**}
G x E	38	292.60 ^{**}	41.62 ^{**}	1.99 ^{**}
Error	120	46.30	21.74	0.09
Total	179			
Overall mean		51.10	46.87	24.37
CV (%)		13.32	9.94	1.22

* $p < 0.05$, ** $p < 0.01$ (Snedecor's F-test). Ns = Not significant. DF = degrees of freedom. CV = coefficient of variation. G x E = Genotype by environment interaction; Fe = iron content; Zn = zinc content; PC = protein content.

Cooking quality

Significant differences were observed between the genotypes, environments, and G x E interaction effects ($p < 0.01$) for the trait CQ (Table 5).

Table 5: Summary of the ANOVA for cooking quality in cowpea evaluated at three environments in the Piauí semi-arid zone of Northeastern Brazil.

Source of variation	DF	Mean squares
		CQ (%)
Block/Environment	3	277668.48
Environment (E)	2	5063382.32 ^{**}
Genotype (G)	19	145237.51 ^{**}
G x E	38	89398.59 ^{**}
Error	57	92861.26
Total	119	
Overall mean		1209.13
CV (%)		25.44

* $p < 0.05$, ** $p < 0.01$ (Snedecor's F-test). Ns = Not significant. DF = degrees of freedom. CV = coefficient of variation. G x E = Genotype by environment interaction; CQ = cooking quality.

It can be inferred that the environments presented a contrast with each other and the differential behavior of the genotypes concerning the variations in the test environments for this trait. The existence of variability among the cowpea genotypes for the cooking time trait was observed. This result demonstrated the possibility

of selecting cowpea genotypes with the fast cooking trait as long cooking time could reduce the bioavailability of the nutrients (Nielsen et al., 1993; Addy et al., 2020). Brazilian consumers prefer cowpea grains with fast cooking because it consumes less cooking gas and time (Freire Filho, 2012).

The percentage of cooked cowpea grains in this study was 48.70, and in other words, half of the evaluated genotypes had adequate cooking time for human consumption (Table 5). Studies on the cowpea cooking time trait were rare in the literature due to its long, expensive, and difficult evaluation technique. Moreover, another factor that could increase or decrease this time was the forms of storage and genetic controlling of the trait, which required further evaluations to understand this trait (Addy et al., 2020).

The results demonstrated that the group of evaluated genotypes in this study had a satisfactory CQ. However, cooking time might depend on the environment where the genotypes were grown as well as the effect of the G x E interaction (Farinelli; Lemos, 2010; Perina et al., 2014). The grains grown in rainy seasons had shorter cooking times as compared with the grains grown under drought conditions, which might be increased this time due to the poor hydration of grains (Perina et al., 2014).

Selection of multi-traits based on the Genotype by Yield*Trait Biplot Approach

The formation of a polygon was observed, resulting from the connection between the genotypes with the longest vertices in all directions (Figure 1).

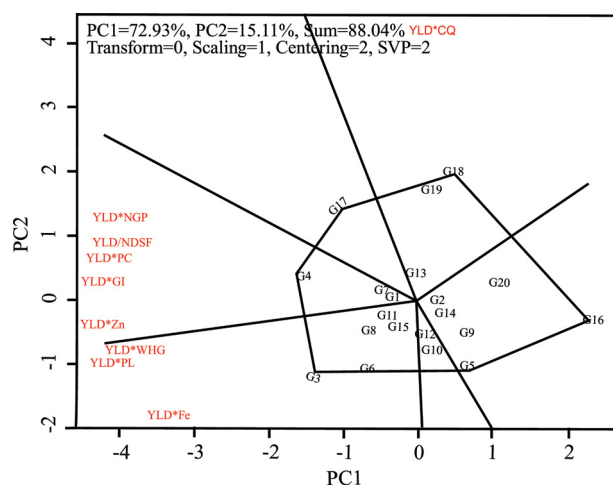


Figure 1: The GYT Biplot “which-won-where” of 20 cowpea genotypes regarding the association between the grain yield and target traits. Biplot based on the decomposition of singular values from the standardized GYT table (“Scaling = 1, Centering = 2”). The singular value partition focused on traits (“SVP = 2”) was used. NDF = number of days to flowering; PL = pod length; NGP = number of grains per pod; WHG = weight of one hundred grains; GI = grain index; YLD = grain yield; Zn = iron content; Fe = zinc content; PC = protein content; CQ = cooking quality.

On each side of the polygon, a line was drawn from the origin of the Biplot dividing it into sectors that represented the profiles of the genotypes concerning the traits (Figure 1). In this context, the genotypes located at each vertex had the highest values for the grain yield-trait combinations. The GYT Biplot analysis showed that the first two components explained almost the totality of the variation among the genotypes (88.04%), demonstrating high reliability.

The polygon showed the formation of six sectors, but only three contained the evaluated traits (Figure 1). At the apex of the first sector, the G18 showed the highest values for the YLD*QC, and the G19 genotype also showed potential for this combination. Therefore, it could be inferred that these two genotypes were the bests for combining the grain yield and cooking quality traits. This combination could be explained due to the cultivars, G18 and G19, which were already commercialized on the market for their good agronomic and culinary characteristics along with their adaptive capacity to the conditions of the semi-arid zone of Northeastern Brazil.

The G4 was located in the sector with the best combinations of YLD*NGP, YLD*NDF, YLD*PC, YLD*GI, and YLD*Zn (Figure 1). On the other hand,

the G3 was positioned at the apex of the third sector showing better combinations of YLD*WHG, YLD*PL, and YLD*Fe, which indicated that this genotype obtained the highest values for the combinations of traits, YLD with WHG, PL, and Fe content.

The G17 was located at the apex of its respective sector, though there was no trait associated with it, which suggested that it did not demonstrate any superior performance for a set of traits but obtained desired behavior because of its location at the apex of the sector where the superior traits were present. The genotype G18, followed by G19, remained isolated in one sector as they showed the highest average values for the CQ in relation to the other genotypes.

According to Paramesh et al. (2016), the which-won-where graph displayed the best genotypes for the evaluated traits at the vertices of the polygon. Thus, both the G4 and G3, followed by the G17, showed promises in combining the production components with the nutritional and cooking quality in cowpea plants. On the other hand, the ones located within the polygon were considered the least sensitive to these traits. The GT Biplot showed the strengths and weaknesses of each genotype by enabling the selection of the parents for possible crosses and considering the similarities and dissimilarities of the genotypes. Both the G1 and G7 behaved similarly for most of the traits, except the GI*CQ combination in the test environments.

The order of genotypes that had the best classifications based on the ability to combine grain yield and target traits were: G4>G3>G17>G8>G6>G7>G11>G1>G13>G15. On the other hand, the G16, G20, and G5 were considered the poorest ones in comparison to the others (Figure 2). The G4, G3, and G17 were considered the most balanced ones for several traits. Moreover, the G7 and G1 were considered the most stable for the most traits, except for the CQ. The G6 had higher values of the IC, PL, WHG, and Zn but lower values of the CQ. However, the G19 and G18 showed high levels of CQ but low performance in the set of other traits. In addition, the genotypes that were below the tester’s median axis (ATA) tended to have good levels of GI, Zn, WHG, PL, and Fe, whereas the opposite occurred with the genotypes located close to the ATA.

According to the GYT superiority index (IS), the classified genotypes based on the ranks containing the average of all trait combinations (Table 6), the genotypes with the highest IS values were the lines G4, G3, G17, G6, and G7. However, the line G16, the cultivar G20, and the line G5 were identified as the poorest genotypes for multiple traits.

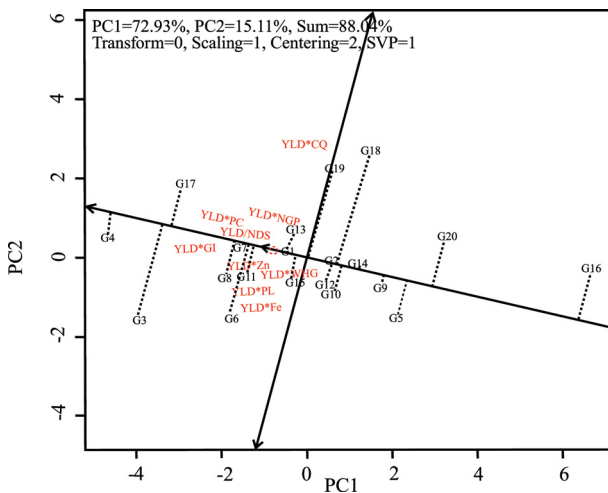


Figure 2: The GYT Biplot representing the mean vs. trait indicating the superiority rank of genotypes. The Biplot was based on the decomposition of singular values from the standardized GYT table (“Scaling = 1, Centering = 2”). The singular value partition focusing on the genotype (“SVP = 1”) was used. NDF = number of days to flowering; PL = pod length; NGP = number of grains per pod; WH = weight of one hundred grains; GI = grain index; YLD = grain yield; Fe = iron content; Zn = zinc content; PC = protein content; CQ = cooking quality.

Table 6: Standardized data for the Genotype by Yield*Trait (GYT) Biplot and superiority index of 20 cowpea genotypes evaluated at three environments in the Piauí semi-arid zone of Northeastern Brazil.

Genotype	Superiority index GYT	Genotype	Superiority index GYT
G1	0.22	G11	0.32
G2	-0.09	G12	-0.10
G3	1.41	G13	0.10
G4	1.63	G14	-0.37
G5	-0.83	G15	0.01
G6	0.74	G16	-2.49
G7	0.59	G17	1.25
G8	0.46	G18	-0.53
G9	-0.69	G19	-0.16
G10	-0.30	G20	-1.19

The cowpea lines showing the highest superiority indexes for all the evaluated traits were needed to be validated in the environmental conditions of at least one more crop year to improve the precision in the selection and recommendation as new cultivars.

The selection based on multiple traits can produce balanced outcomes with the selection. Two studies that evaluated cowpea genotypes in Cerrado (the Brazilian tropical savanna), based on multiple traits, identified the superior lines for most of the combinations between yield and the key traits during the cowpea breeding through the GYT Biplot (Oliveira et al., 2019; Cruz et al., 2020; Araújo et al., 2021). The use of the GYT index in selecting multiple traits became a promising strategy as compared to the traditional indexes. The differential of this approach allowed the selection of the most yielding genotypes associated with the other evaluated traits (Yan et al., 2019).

CONCLUSIONS

The cowpea lines, MNC11–1013E-35 and MNC11–1013E-15, were the superiors because they combined the grain yield trait simultaneously with the yield and nutritional components traits during the cultivation in the Piauí semi-arid zone. However, MNC11–1052E-3 was better in the combination of yield and cooking quality than the other.

ACKNOWLEDGEMENTS

We acknowledge support from the Embrapa Meio-Norte and HarvestPlus Program (Projects No.20.18.01.022.00.00 and 20.19.00.118.00.00) for funding this work. We also thank to Fundação de Amparo à Pesquisa do Estado do Piauí (FAPEPI), Universidade Federal do Piauí and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting of Scholarship to the first author (Grant 88887.200984/201800).

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