Combining ability of cowpea genotypes for green grain production

Angela Maria dos Santos Pessoa[1*], *Cândida Hermínia Campos de Magalhães Bertini*[2], *Érika Beatriz de Lima Castro*[2], *Leslyene Maria de Freitas*[2], *Tomil Ricardo Maia de Sousa*[2]

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ABSTRACT

Cowpea is a species used for various purposes, including green grain production. New cowpea cultivars should be developed to answer the growing market demands for green gains, which can be achieved via breeding programs. In this scenario, diallel cross is a breeding method that allows researchers to select the most suitable parents for hybridization. From this perspective, this study aimed to identify cowpea parents and hybrids with good combining abilities to initiate a breeding program aimed at green grain production. The experiment was developed at the Plant Science Department of the Federal University of Ceará. Fifteen treatments (five parents and ten hybrids) were set up in a randomized block design with ten replications. The variables of green pod length, green pod mass, number of grains, grain mass per green pod, and green grain length were studied in this experiment. The data were subjected to analysis of variance, test of means, and diallel analysis. The parents and hybrids showed variations for the evaluated traits. Both additive and non-additive effects influence the behavior of inherited traits. The parent CE0002 and hybrid combinations BRS Juruá x CE1002 and CE0957 x CE0002 are recommended for selection, showing desirable aspects for green grain production.

Keywords: diallel; hybrids; plant breeding; *Vigna unguiculata*.

INTRODUCTION

Vigna unguiculata (L.) Walp., commonly known as cowpea, is a plant species of the family Fabaceae that shows high contents of minerals and proteins, low contents of lipids, and can be used to combat malnutrition in several developing countries (Rengadu *et al.*, 2020). Cowpea is one of the growing food sources in tropical and subtropical regions (Kebede & Bekeko, 2020), especially in Africa, Latin America, part of Asia, and in the United States of America (Xiong *et al.*, 2016; Narli & Ozcan, 2022), where it is used as a protein source for humans (Amusa *et al.,* 2021) and animals (Amusa *et al.*, 2021). This species can be consumed as dry grains (Tomaz *et al.*, 2022), as a source of starch for the food industry (Acevedo *et al.*, 2022), or as green grains (Aquino *et al.*, 2021).

In this scenario, the growing increase of the green bean market has broadened the global demand for new cultivars. However, developing new cowpea genotypes for green grain production depends on overcoming challenges such as the scarce available information about this species (Aquino *et al.*, 2021). The prospection for developing new cowpea varieties consists of combining desirable alleles from various previously selected germplasms and recombining them to obtain superior genotypes (Rocha *et al.*, 2014; Reyes *et al.*, 2022).

In this context, the diallel cross method is one of the ways to obtain segregating populations and select the best individuals to obtain pure cowpea lines (Torres *et al.*, 2021). This procedure allows researchers to determine which

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[1] Universidade Federal Rural do Semi-Árido, Mossoró, RN, Brazil. angela.pessoa@ufersa.edu.br
[2] Universidade Federal do Ceará, Fortaleza, CE, Brazil. candida@ufc.br; erika-beatriz@hotmail.com; freitaslesly61@gmail.com; tomilsousa@ufc.br
*Corresponding author: angela.pessoa@ufersa.edu.br

parents can be selected, identify heterotic effects, assess the genotype's performance, and the effects of general and specific combining abilities. The general combining ability refers to the average parental performance in crosses. In contrast, the specific combining ability relates to the performance of the best or worst hybrid based on the average performance of the parents involved in the hybridization (Chen *et al.*, 2019).

The genetic variability in cowpea accessions reported by Pessoa *et al.* (2022) for the Active Cowpea Germplasm Bank (BAG) of the Federal University of Ceará (UFC), in northeastern Brazil, has highlighted the potential of these genotypes for green grain production. From this perspective, given the results obtained in previous research and the demand for new green grain cultivars, this study aimed to identify cowpea parents and hybrids with good combining abilities that could assist in identifying promising genotypes to initiate a breeding program aimed at the production of cowpea green grains.

MATERIAL AND METHODS

Location of the experiment and plant material

The experiment was conducted in the experimental area belonging to the Horticulture Sector of the Plant Science Department of the Center of Agricultural Sciences (CCA) of the Federal University of Ceará (UFC), Campus Pici (3º 44' 27,6" S, 38º 34' 38,5" W), in Fortaleza - Ceará, Brazil.

The plant material was obtained from the Active Cowpea Germplasm Bank at UFC, consisting of four genotypes (UFC-02, UFC-957, UFC-999, and UFC-1002) and one commercial cultivar (BRS Juruá) (Table 1), selected based on their genetic variability for morpho-agronomic traits (Pessoa *et al.*, 2022), in addition to 10 hybrids resulting from crosses between these parents.

Diallel crosses and field experiment for populations

Five genotypes were chosen as parents and crossed in diallel by omitting the reciprocals and considering the genetic distance and desirable morpho-agronomic traits.

The seeds of the cowpea parents were sown at different moments to synchronize flowering and then perform the crosses. The seeds were sown in 30-L pots containing soil (90%) and humus (10%) and irrigated daily under plant nursery conditions from August 2020 to March 2021 at the Horticulture Sector of the Plant Science Department of UFC.

For the crosses, pollen samples were collected in the morning (open flowers) and preserved in a refrigerator. Then, the flowers were emasculated and pollinated in the late afternoon, 12 to 14 hours before their natural opening (Zary & Miller Junior, 1982). The hands and tweezers used in the procedure were sanitized with 90% ethyl alcohol. Then, the pollinated flowers were tagged and identified with the date and name of the parents. Finally, the dry pods were harvested, and the F_1 seeds were collected, processed, and stored.

The population was composed of five parents and ten hybrids, totaling 15 treatments. The total experimental area was 333 m^2, with each block measuring 111 m^2, formed by three central rows 18.8 m long and a two-meter space between blocks. Chemical weed control was performed with glyphosate. Fertilization was performed based on chemical analysis (Table 2) and considering the crop requirements (Freire Filho, 2011). The spacing adopted was 1.0 m between rows and 0.50 m between plants in the row. Each genotype was represented by ten plants, with two plants per hole. Three seeds were sown per hole, and the plants were thinned to two per hole 15 days after sowing.

Table 1: Morpho-agronomic markers of the parents used in the crosses

ID BAG	Class/subclass	DFL	PPL	CFL	PAV
CE0002	Colored/ 'manteiga'	45	Semi-prostrate	Violet	Absent
CE0957	White/ 'branco liso'	42	Erect	White	Absent
CE0999	White / 'fradinho'	42	Erect	White	Absent
CE1002	White / 'branca'	38	Erect	White	Absent
BRS Juruá	Colored / 'verde'	44	Semi-prostrate	White	Present

DFL – Days to flowering (mean values of 18 plants); PPL – Growth habit; CFL – Flower color; PAV – Presence of anthocyanin in the pods.

Table 2: Chemical attributes of the soil in the experimental area

pH	O.M.	P	K^+	Na^+	H^++Al^{3+}	Al^{3+}	Ca^{2+}	Mg^{2+}	BS	CEC
	(g kg^{-1})	(Mg dm^{-3})				(cmol$_c$ dm^{-3})				
7.6	5.21	34.15	35.33	0.04	0.00	0.00	1.54	1.02	2.69	2.69

OM – Organic matter; BS – Base saturation; CEC – Cation Exchange capacity.

The crop management practices consisted of weed control by hoeing, which was performed close to flowering. Insecticides were applied to control pests during plant development (15 and 30 days after emergence, Decis® 25 EC). A sprinkler irrigation system was set up to irrigate the plants once a day early in the morning by maintaining soil moisture between 70 and 90% of field capacity.

Data collection and statistical analysis

The traits evaluated were: green pod length [(GPL- cm) determined as the mean of ten pods per treatment using a ruler]; green pod mass [(GPM-g) determined as the mean weight of ten green pods]; number of grains [(NG) determined by counting the number of grains per pod]; grain mass per green pod [(GMP-g) determined by weighing the grains of each pod considering the mean of ten pods]; and green grain length [(GGL-cm) obtained using a digital caliper considering ten grains].

A randomized block design with 15 treatments (parents and hybrids) and ten replications per treatment was used in the experiment. The data were subjected to analysis of variance, followed by the Scott-Knott test of means ($p \leq 0.01$ or $p \leq 0.05$).

An analysis of variance was initially performed based on the mean of the plots for the evaluated traits and considering all effects as fixed, except the block and experimental error (fixed model). The general combining ability (GCA) and the specific combining ability (SCA) were estimated based on the methodology proposed by Griffing (1956), method 2, adapted for partial diallel cross using parents and hybrid combinations according to the following model:

$Y_{ijk} = \mu + g_i + g_j + s_{ij} + e_{ij}$, where: Y_{ijk} is the observation value of a cross between the i-th and j-th parents; μ = population mean; g_i and g_j are the GCA values of parents ith and jth, respectively; s_{ij} is the SCA value for the hybrid between parent ith and jth; μ – is the effect of the overall mean; G_i and G_j – is the effect of the general combining ability (GCA) associated with the i and j-th parent, and e_{ij} – eijk is the experimental error. All statistical analyses were performed with the software Genes (Cruz, 2016).

RESULTS

Clustering of means

According to the Scott-Knott test, the parents and hybrids showed variations for the evaluated traits (Table 3).

The number of grains per pod and the grain mass per green pod showed the highest variation, forming three different classes (Table 3). The parent CE0002 and the hybrid combinations BRS Juruá x CE0002 and BRS Juruá x CE1002 showed the highest performances for these traits.

The greed pod length, green pod weight, and green grain length formed two different classes. Parents CE0002 and CE0999 and hybrids CE1002 x CE0002, CE0957 x CE0002, CE0999 x CE0002, BRS Juruá x CE0002, BRS Juruá x CE1002, and BRS Juruá x CE0957 showed the highest mean values for these traits (Table 3).

Combination ability

The analysis of variance revealed significant differences between treatments for all variables (Table 4).

The effects of the general combining ability (GCA) were significant for all evaluated traits according to the F-test ($p < 0.01$) (Table 4). The specific combining ability (SCA) showed significance for the number of grains per pod, grain mass per pod, and green grain length ($p < 0.01$ and $p < 0.05$).

The green pod length and green grain length showed high and positive values for the quadratic component related the GCA (Table 4). However, the SCA showed a significant effect for the green pod mass, number of grains per pod, and grain mass per green pod.

The genotype CE0002 showed positive and significant GCA values for the green pod length, green pod mass, number of grains, and grain mass per green pod (Table 5). For most traits studied, genotypes CE1002 and CE0957 stood out with significant and negative values.

The hybrid combination BRS Juruá x CE1002 showed significant and positive values for all traits (Table 6). The performance of this hybrid combination was also observed in the mean values (Table 3).

Table 3: Means of quantitative traits in cowpea parents and hybrids for green grain production

Parents/hybrids	Traits				
	GPL(cm)	GPM(g)	NG(unit)	GMP(g)	GGL(cm)
CE0002	27.46a	9.83a	13.03a	5.76a	1.43a
CE1002	14.99b	4.63b	6.56c	2.09c	1.10b
CE0957	16.50b	5.76b	7.58c	2.55c	1.16b
CE0999	21.33a	8.74a	9.43b	3.23c	1.23a
BRS Juruá	18.10b	6.70b	9.41b	2.71c	1.16b
CE1002 x CE0002	21.59a	8.85a	9.66b	3.86b	1.25a
CE0957 x CE0002	23.31a	11.13a	9.93b	4.44b	1.36a
CE0999 x CE0002	22.70a	12.14a	9.94b	4.33b	1.37a
BRS Juruá x CE0002	23.24a	9.64a	12.50a	5.55a	1.32a
CE0957 x CE1002	15.86b	5.35b	6.21c	2.22c	1.13b
CE0999 x CE1002	19.88a	7.41b	9.83b	2.61c	1.08b
BRS Juruá x CE1002	12.24b	9.64a	12.50a	5.55a	1.32a
CE0999 x CE0957	16.87b	6.84b	7.90c	2.38c	1.08b
BRS Juruá x CE0957	21.53a	9.35a	10.60b	4.03b	1.24a
BRS Juruá x CE0999	20.07a	7.35b	7.90c	3.21c	1.28a

Green pod length (GPL), green pod mass (GPM), number of grains (NG), grain mass per green pod (GMP), and green grain length (GGL). Means followed by the same letter in the column do not differ statistically by the Scott-Knott test (p = 0.05).

Table 4: Analysis of variance (mean squares) and quadratic components associated with the effects of the general combining ability (φ^2_g) and specific combining ability (φ^2_s) for quantitative cowpea traits evaluated for green grain production

Source of variation	DF	Traits				
		GPL(cm)	GPM(g)	NG(unit)	GMP(g)	GGL(cm)
Treatments	14	35.73**	13.96*	13.22**	4.83**	0.03**
GCA	4	89.23**	27.18**	26.71**	10.57**	0.08**
SCA	10	14.34^{ns}	8.67^{ns}	7.82*	2.54**	0.01*
Residual	30	8.54	5.42	3.38	0.38	0.01
ϕ^2_g	-	3.84	1.03	1.11	0.48	0.03
ϕ^2_s	-	1.93	1.08	1.47	0.71	0.02
ϕ^2_g / ϕ^2_s	-	1.98	1.00	0.75	0.67	1.00

^{ns}Non-significant ** and * significant at 1 and 5% of probability by the F-test. Degrees of freedom (DF), green pod length (GPL), green pod mass (GPM), number of grains (NG), grain mass per green pod (GMP), and green grain length (GGL).

Table 5: Estimates of the general combining ability (GCA) referring to quantitative cowpea traits evaluated for green grain production

Parents	Traits				
	GPL(cm)	GPM(g)	NG(unit)	GMP(g)	GGL(cm)
CE0002	3.30**	1.72*	1.58*	1.12**	0.11**
CE1002	-1.73*	-1.26*	-0.82^{ns}	-0.48**	-0.06**
CE0957	-1.73*	-0.73^{ns}	-1.08*	-0.52**	-0.04*
CE0999	-0.06^{ns}	0.26^{ns}	-0.42^{ns}	-0.40*	-0.02^{ns}
BRS Juruá	0.22^{ns}	0.04^{ns}	0.74^{ns}	0.27^{ns}	0.01^{ns}

^{ns}Non-significant, ** and * significant at 1 and 5% of probability, respectively, by Student’s t-test. Green pod length (GPL), green pod mass (GPM), number of grains (NG), grain mass per green pod (GMP), and green grain length (GGL).

Table 6: Estimates of the specific combining ability (SCA) referring to quantitative cowpea traits evaluated for green grain production

Parents/hybrids	Traits				
	GPL(cm)	GPM(g)	NG(unit)	GMP(g)	GGL(cm)
CE1002 x CE0002	-0.43ns	0.16ns	-0.60ns	-0.41ns	-0.03ns
CE0957 x CE0002	1.28ns	1.91ns	-0.08ns	0.20ns	0.05*
CE0999 x CE0002	-0.90ns	1.92ns	-0.73ns	-0.03ns	0.04ns
BRS Juruá x CE0002	-0.74ns	-0.31ns	0.65ns	0.50ns	-0.03ns
CE0957 x CE1002	-1.12ns	-0.87ns	-1.40ns	-0.41ns	-0.01ns
CE0999 x CE1002	1.22ns	0.18ns	1.56ns	-0.13ns	-0.07*
BRS Juruá x CE1002	4.29*	2.66*	3.05*	2.11**	0.13**
CE0999 x CE0957	-1.78ns	-0.91ns	-0.40ns	-0.33ns	-0.09*
BRS Juruá x CE0957	2.58ns	1.85ns	1.42ns	0.63*	0.03ns
BRS Juruá x CE0999	-0.53ns	-1.15ns	-1.94ns	-0.30ns	0.05*

nsNon-significant, **and* significant at 1 and 5% of probability, respectively, by Student's t-test. Green pod length (GPL), green pod mass (GPM), number of grains (NG), grain mass per green pod (GMP), and green grain length (GGL).

For the grain mass per green pod and green grain length, in addition to the hybrid do BRS Juruá x CE1002, the hybrid BRS Juruá x CE0957 stood out for the grain mass per green pod, and the combinations CE0957 x CE0002 and BRS Juruá x CE0999 stood out for green grain length (Table 4), with positives and significant values.

DISCUSSION

Clustering of means

The variability identified among cowpea genotypes for grain and pod traits by the test of means was also reported by other authors for dry grains (Gomes Filho *et al.*, 2017; Gomes *et al.*, 2018), with scarce information available about green grains. Although the commercialization of immature cowpea seeds (green grains) is expressive in the Northeast region, there are no recommendations of cultivars identified for this specific purpose (Aquino *et al.*, 2021). In this scenario, the presence of variability is essential to identify superior genotypes.

The greatest variations in the mean values referring to the number of grains per pod and the grain mass per green pod indicate that the genotypes have grains of different sizes. Seed classification by size and mass could be a strategy to increase the yield since seed size affects grain production (Araújo Neto *et al.*, 2014).

For the green pod length, green pod weight, and green grain length, genotypes with the highest mean values are recommended for selection, highlighting the potential of these genotypes in providing benefits for breeding programs aimed at developing cultivars for green grain production. Genotypes from germplasm banks that had not yet been genetically improved, such as these accessions, can show variability for morphological seed parameters and successfully used for breeding (Drun *et al.*, 2017), depending on the purposes of selection.

Combination ability

The significant differences identified in the analysis of variance could be attributed to the existing variability between the parents and hybrids used in this study, enabling the selection of plants with desirable traits. Diallel crosses constitute a viable alternative to widen the genetic base of cowpea and obtain superior populations (Rodrigues *et al.*, 2018). Al-Obeidi *et al.* (2022) also reported variability in pod and seed traits of cowpea parents and hybrids, indicating that the genotypes are genetically different and that different genes control the inheritance of traits.

The significant effects of the general combining ability (GCA) indicate that the parents differed in the frequency of favorable alleles, with some being more promising than others in the formation of superior populations.

The significance of both traits based on the GCA and SCA indicates that additive and non-additive genetic effects play a dominant role in expressing these traits (Lang *et al.*, 2022).

The estimates of the quadratic components for the GCA with high and positive values (pod length and grain length) indicate that additive effects are involved in the genetic control of these traits, which can be effectively selected in the first segregating populations (Badhe *et al.*, 2016; Purnamasari *et al*., 2019) or even to decrease or increase a specific trait by backcrossing (Pessoa *et al.*, 2019), favoring genetic improvement through selection.

The quadratic component estimates for SCA with high and positive values indicate the predominance of non-additive genetic effects of dominance and/or epistasis for the expression the traits, as reported by Al-Mamun *et al.* (2022), according to the results observed in the green pod mass, number of grains per pod, and grain mass per green pod.

Genotype CE0002, showed positive and significant values for the evaluated traits, highlighting its superiority and the potential for use in breeding programs aimed at developing cultivars for green grain production. In Northeastern Brazil, green beans are marketed as grains and pods, with consumers usually preferring large pods and grains, justifying the importance of studies such as the present research. Sopan *et al.* (2018) reported that parents with high, significant, and positive GCA values are good combinators for the studied traits and are considered sources of favorable alleles for breeding programs.

Genotypes with significant and negative GCA values (CE1002 and CE0957) are recommended for futures crosses to obtain segregating populations of plants with small pods and grains, which could be an alternative for other studies on this species. Parent selection for breeding programs can be performed through diallel crosses using the general combining ability, with negative and high-magnitude GCA estimates for a given trait, contributing to reducing its expression (Rodrigues *et al.*, 2018), as observed for these parents.

The hybrid combination BRS Juruá x CE1002 can be recommended for selection due to the significant and positive values observed for the evaluated traits, indicating that dominant genes contributed to increasing their expression. Selection based on the specific combining ability should be based on the highest positive or negative SCA according to the importance of the trait, provided that the parents have a high GCA (Griffing, 1956). However, the commercial production of hybrids is not recommended for cowpea, , first being necessary to evaluate hybrids and select those with the best performance based on the GCA to advance generations.

Even if few hybrids showed significance, hybridization is a strategic procedure of extreme importance in cowpea breeding programs aimed at obtaining segregating populations and advancing generations. However, hybridization in this species faces several obstacles. Amusa *et al.* (2021) reported that high temperatures reduce fertility, whereas low temperatures delay the initiation of the pollen tube and result in a short duration of flower receptivity, thus resulting in failure during seed or pod formation.

CONCLUSIONS

According to Griffing's diallel analysis, the cowpea genotype CE0002 is a good parent for green grain production based on pod and seed traits and can be used to compose new hybrid combinations in breeding programs of this species.

The hybrid combinations BRS Juruá x CE1002, CE0999 x CE0002, and CE0957 x CE0002 are recommended to advance generations and obtain cowpea plants with desirable traits for green grain production.

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