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Original article

Cowpea: A low-cost quality protein source for food safety in marginal areas for agriculture

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ABSTRACT

Cowpea is a low-cost protein source for human nutrition for the world's impoverished regions. Therefore, the yield and total grain protein content (TGPC) of two modern commercial genotypes, Novaera and Gurguéia, and two traditional local genotypes, Paulistinha and EPACE-10, were studied. Also, leaf area and dry weight, leaf soluble protein content, and chlorophyll *a* fluorescence, parameters related to photosynthetic capacity, were used to evaluate genotypes. Under optimal conditions, the yield of EPACE-10 and Paulistinha, with higher TGPC, was lower than for Gurguéia and Novaera, which showed lower TGPC. The four cowpea genotypes showed high lysine content and low methionine and cysteine. The results revealed a negative correlation between yield and TGPC. The modern commercial genotype Novaera showed a high yield with low TGPC but a higher globulin and albumin content than Gurguéia. Thus, it can be used in high-input agriculture. In contrast, the traditional local genotype EPACE-10, with high TGPC and higher amino acid content than Paulistinha, is indicated for low-input agriculture in marginal areas for food safety under climate changes.

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1. Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is an important pulse crop that serves multiple purposes: for human food, animal feed, as green manure and others uses (Freire Filho et al., 2005; Jayathilake et al., 2018). The plant is efficient at biological nitrogen fixation and do not need nitrogen fertilization to have a good yield (Pimentel et al., 1999b; Silva Júnior et al., 2012). It is one of the pulses best adapted to environmental stresses (Alghamdi et al., 2019), as drought (Pimentel et al., 1999b), salinity (Farooq et al., 2020), and high temperature (Costa et al., 2002). In general, it is produced by family farming in low-input agriculture, without fertilization or irrigation (Freire Filho et al., 2005; Jayathilake et al., 2018), in marginal areas for agriculture (FAO, 2017), where soils are deficient in nutrients, and environmental stresses are frequent

(Farooq et al., 2020; Pimentel, 2006). As population growth increases, especially in these marginal regions (FAO, 2017), the primary demand seems likely to outpace food production (FAO/IFAD/UNICEF/WFP/WHO, 2020; Fasolin et al., 2019). Therefore, in the future, under increasing climate changes, food safety in these marginal areas will need to be based on less expensive vegetable protein from crops more adapted to environmental stresses (Farooq et al., 2020; Fasolin et al., 2019).

In marginal areas for agriculture, cowpea is produced by small farmers using traditional local genotypes (FAO, 2017). These local genotypes (also called landraces) will have a lower yield in optimal conditions, but they are better adapted to abiotic stresses so frequent in these areas (Freire Filho et al., 2005; Jayathilake et al., 2018). The cowpea yield potential is attained only in high-input agriculture, with modern commercial genotypes more dependent on fertilization, pesticides, and irrigation (Alghamdi et al., 2019; Freire Filho et al., 2005; Vasconcelos et al., 2010).

Cowpea grain contains an average of 23–32 % grain protein, 50–60 % carbohydrate, and about 1 % fat on a dry basis (Jayathilake et al., 2018). Among the grain proteins, globulins contain up to 52 % of essential amino acids, albumins contain up to 44 %, glutelins contain around 30 %, and prolamins contain up to 22 % (Lookhart and Bean, 2000). Prolamins and glutelins are the main

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grain proteins in cereals, whereas, in pulses, globulins and albumins are in greater quantity (Petsko and Ringe, 2004). Therefore, cowpea proteins contain more lysine than sulfur-containing amino acids, such as methionine and cysteine (Elhardallo et al., 2015). Consequently, for food safety, cowpea is a complement for cereals, which have low amounts of lysine but are richer in methionine and cysteine (Fasolin et al., 2019; Alghamdi et al., 2019).

However, selection to increase total grain protein content (TGPC) can decrease seed weight and yield in corn (Dudley and Lamber, 2004) and wheat (Faměra et al., 2015). Nevertheless, genetic differences in N assimilation and utilization exist between cultivars, and some wheat cultivars consistently accumulate higher protein contents than expected based on their yields (Chope et al., 2014). An increase in photosynthesis during the pre and flowering stages (Pimentel et al., 1999a) or leaf senescence and nitrogen remobilization during grain growth can favor protein deposition over starch accumulation (Rharrabati et al., 2001).

The primary purpose of this study is to recommend cowpea genotypes to produce a low-cost quality protein to nourish the increasing population in marginal areas for agriculture. Thus, this work aims to evaluate some physiological parameters associated with photosynthesis and relate them to the yield, grain protein content, and quality of four genotypes and correlate them. These physiological parameters were successfully used to discriminate cowpea adaptation to water deficit (Pimentel et al., 1999b) and high temperature (Costa et al., 2002). Two high-yielding modern commercial genotypes indicated for high-input agriculture (Vasconcelos et al., 2010) and two low-yielding traditional local genotypes cultivated in low-input agriculture (Gonçalves et al., 2020) will be the object of study.

2. Materials and methods

2.1. Plant material and growth conditions

Four cowpea genotypes with different yield potentials and sensitivity to abiotic stresses were cultivated in Seropédica, RJ, Brazil (22°45' S, 43°41' W) under greenhouse conditions between August and December 2018, harvest 115 days after sowing (DAS). During the experiment, the air temperature varied between 20 and 33 °C, and the pots were irrigated every morning to reach saturation.

The genotypes used were: Gurguéia and Novaera, two modern commercial genotypes recently launched by the Brazilian Agricultural Research Corporation (EMBRAPA), with high potential yield for use in high-input agriculture by big farmers; and EPACE-10, a traditional local genotype selected for use by small farmers without high technology, well adapted to drought and high temperature (Pimentel et al., 1999b; Costa et al., 2002), and Paulistinha, another traditional local genotype selected in a marginal area under high temperature and water deficit (Freire Filho et al., 2005; Gonçalves et al., 2020) (Table 1). The analyses were performed in four distinct phenological stages: vegetative, pre-flowering, flowering, and pod filling. The trial was conducted in a Kanhapudalf soil, with the following composition at a depth of

0.2 m: pH 5.0, 18 mM Ca, 8 mM Mg, 2 mM Al, 0.8 mM available P, 2 mM available K, and 10.7 g kg⁻¹ of organic matter. Before sowing, all the seeds were inoculated with *Bradyrhizobium* strain BR3262 (SEMIA 6464) recommended by the Brazilian Agricultural Research Enterprise (EMBRAPA) (Silva Júnior et al., 2012). Two plants were grown in each pot containing 10 kg of soil, which was fertilized with an equivalent of 1.5 t CaCO₃ ha⁻¹, 60 kg P₂O₅ ha⁻¹, and 40 kg K₂O ha⁻¹, without N fertilization, according to recommendation (Freire Filho et al., 2005; Silva Júnior et al., 2012).

2.2. Physiological parameters associated with photosynthesis

At each of the four phenological stages, six plants from three pots were collected without the roots to evaluate the leaf area, shoot dry weight, LSPC, and chlorophyll *a* fluorescence emission.

2.2.1. Leaf area and shoot dry weight

The leaf area was measured with a portable area meter LI-3000C (LICOR, USA), and shoot dry weight was obtained after drying at 65 °C for 72 h.

2.2.2. Leaf soluble protein content analysis

The central leaflet of the youngest fully expanded leaf of six different plants was collected in each of the four phenological stages to quantify LSPC using the Bradford method (Bradford, 1976).

2.2.3. Chlorophyll *a* fluorescence analysis

Chlorophyll *a* fluorescence measurements were always made on the same central leaflet, as per the LSPC analysis, but of another youngest fully expanded leaf, using a Mini-PAM modulated fluorometer (Heinz Walz, Effeltrich, Germany). The maximum (F_m) and minimum (F_o) fluorescence were measured in dark-adapted leaves after sundown, as proposed before (Pimentel et al., 2005). F_o was measured on leaves after their adaptation to the dark, for at least 30 min, under low and modulated illumination (<0.5 μmol m⁻² s⁻¹), and F_m was measured after a pulse of light saturation (18,000 μmol m⁻² s⁻¹) lasting 3 s. From these measurements, the yield of the fluorescence variable was calculated (F_v = F_m - F_o) to obtain the maximum dark-adapted quantum yield efficiency of photosystem II (PSII) (F_v/F_m = (F_m - F_o)/F_m) (Schreiber et al., 1994). In light-adapted leaves, under 500 and 1000 μmol m⁻² s⁻¹ of photosynthetic photon flux density (PPFD), the effective and actual quantum yield of PSII (Φ_{PSII} = F_m' - F_t/F_m') was measured (Murchie and Lawson, 2013). These measurements on light-adapted leaves were done at 10 a.m., when A is maximal (Pimentel et al., 1999a), and calculated as Φ_{PSII} = (F_m' - F_t)/F_m'; F_m' being the maximum fluorescence after light exposure and F_t the transitory fluorescence. Therefore, the chlorophyll *a* fluorescence parameters calculated were: the potential maximum quantum yield of PSII (F_v/F_m) and the actual effective quantum yield of PS II (Φ_{PSII}) (Schreiber et al., 1994; Murchie and Lawson, 2013).

Table 1
Main characteristics of *Vigna unguiculata* (L.) Walp genotypes used in this work.

Genotypes	Origin/Crossing	Growth habit	Cycle(days)	Grain color	Yield (kg ha ⁻¹)
BR17 Gurguéia	BR10 Piauí x CE-315	semi-branched	75	greenish	900 to 1500
BRS Novaera	TE97-404-1F e TE97-404- 3F	semi-erect	65 to 70	white	1074
Epace 10	Seridó x TVu 1888.	semi-branched	65 to 75	brown	1000
Paulistinha	Local genotype/Juazeiro do Norte - CE	Semi-branched	65 to 75	light brown	1070

Data was obtained from Freire Filho et al. (2005).

2.3. Grain protein extraction and analysis

Grain samples harvested at the maturation of plants, 115 DAS, were used to extract the reserve proteins. The grain samples were ground and lyophilized for flour production that was later used to analyze reserve proteins and amino acids.

Protein fraction extractions were performed using centrifuge tubes (2.0 mL capacity) with 0.3 g of flour and 1 mL of solvent at each step, as previously described (Gonçalves et al., 2020). Centrifugations were performed at 12,000 g for 5 min (Centrifuge 2 K15, Sigma, Germany). The sequential protein fraction extraction used was as follows: a) two steps of defatting with hexane, b) two globulins extractions with 0.5 mol/L NaCl, c) two albumins extractions with distilled water, d) one prolamins extraction with 55 % 2-propanol (w/w) + 0.6 % 2-mercaptoethanol (v/v), e) two glutelin extractions with 0.5 % dodecyl sulfate sodium (w/v) and 0.6 % 2-mercaptoethanol (v/v) in sodium borate buffer pH 10 (0.0125 mol/L Na₂B₄O₇ · 12 H₂O and 0.02 mol/L NaOH) (Vasconcelos et al., 2010). The centrifugations for defatting and extraction of prolamins and glutelins were performed at room temperature, 25 °C. In contrast, the extractions for albumins and globulins were performed at 4 °C (Gonçalves et al., 2020; Vasconcelos et al., 2010). The protein supernatant was retrieved after centrifugation. When two centrifugations were performed, both supernatants were combined and stored at -80 °C. Each protein fraction content was determined according to the Bradford method (Bradford, 1976).

2.4. Grain amino acid analysis

EMBRAPA Food Technology performed the extraction and analysis of amino acids from grains. The analysis of amino acids was done on the lyophilized flour produced for protein extraction. It was done using a liquid chromatograph, model Alliance 2690/5, with column oven and fluorescence detector 2475 (Waters, USA), with a chromatographic Symmetry C18 3.5 µm column (4.6x75mm). The measurements were done according to the methods proposed by AOAC 994.12/2000, as previously described (Liu et al., 1995).

2.5. Electrophoretic SDS-1D-PAGE profiles

The electrophoretic analysis was performed under denaturing conditions (0.1 % (w/v) SDS) in 13 % polyacrylamide gels. For albumins, 3.8 µg of proteins were loaded onto each lane, and 5.0 µg for the other storage protein fractions, under the running conditions described (Schimidt et al., 2015). The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to generate denaturing electrophoresis profiles in a Mini-PROTEAN 3 system running module (BIO-RAD, USA). The lanes were stained with a solution of silver nitrate (Blum et al., 1987; Schmidt et al., 2015).

2.6. Yield components

At physiological maturity, all plants were harvested to determine the number of pods per plant, grains per pod, and grain weight per plant.

2.7. Statistical analysis

The experimental design was completely randomized with four cowpea genotypes × four sampling phenological stages × three replications, analyzing the two plants per pot in each repetition. The analysis of variance was performed with the F test for each quantitative trait. When the treatments presented significance,

means were compared and segregated using the Student-Newman-Keuls (SNK) test with a significance level of $p < 0.05$.

3. Results

3.1. Leaf area, shoot dry weight, and LSPC

Novaera presented significantly higher leaf area values during the vegetative growth stage than the other genotypes (Table 2), followed by Paulistinha with a higher leaf area than Gurguéia and EPACE-10, which had similar results values. Regarding shoot dry weight and LSPC, in this stage, all genotypes showed the same values. There were no significant differences between genotypes in the pre-flowering stage for any of these three parameters studied. In contrast, Paulistinha and EPACE-10 presented the highest leaf area values in the flowering stage compared with Novaera and Gurguéia, which showed similar values; however, no significant differences were detected for the shoot dry weight and LSPC among genotypes at this stage. Finally, at the pod filling stage, significant differences between the genotypes were only observed for LSPC, with Gurguéia and EPACE-10 showing significantly higher values (Table 2). At the same time, the LSPC of Novaera was lower than Gurguéia but similar to EPACE-10 and significantly higher than that of Paulistinha, which showed a significantly lower value than the other three genotypes.

3.2. Chlorophyll α fluorescence

Chlorophyll α fluorescence parameters are shown in Table 3. Regarding the Φ_{PSII} values, at 1000 µmol m⁻² s⁻¹ of PPFD was smaller than at 500 µmol m⁻² s⁻¹. At 500 µmol m⁻² s⁻¹, there was no difference between the Φ_{PSII} values of the genotypes for all stages. However, for the Φ_{PSII} values at 1000 µmol m⁻² s⁻¹ (Table 3), there were significant differences, but only at the pre-flowering stage, when photosynthesis is maximal, with Novaera showing significantly higher values, followed by EPACE-10 and Gurguéia, with the same value, while Paulistinha showed the lowest value among the genotypes. There were differences in the Fv/Fm ratio of the genotypes in the pre-flowering stage when Novaera and Paulistinha showed a significantly higher value. However, the

Table 2

Leaf area, shoot dry weight, and leaf soluble protein content per plant on four cowpea genotypes in four phenological stages.

Genotypes	Leaf area (cm ²)	Shoot dry weight (g)	Leaf soluble protein content (mgBSA gDW)
VEGETATIVE STAGE			
Novaera	147.50 a	0.94 a	2.93 a
Gurguéia	77.93c	0.77 a	2.62 a
Paulistinha	107.73b	0.86 a	2.92 a
EPACE-10	61.76c	0.65 a	2.60 a
PRE-FLOWERING STAGE			
Novaera	399.26 a	3.57 a	3.13 a
Gurguéia	560.33 a	3.62 a	2.87 a
Paulistinha	536.36 a	4.04 a	2.79 a
EPACE-10	419.07 a	2.30 a	2.86 a
FLOWERING STAGE			
Novaera	521.76b	6.49 a	3.26 a
Gurguéia	567.21b	4.87 a	3.43 a
Paulistinha	747.73 a	6.52 a	3.12 a
EPACE-10	792.82 a	5.27 a	3.55 a
POD FILLING STAGE			
Novaera	475.87 a	5.40 a	1.81b
Gurguéia	487.63 a	5.56 a	2.19 a
Paulistinha	416.89 a	5.75 a	1.47c
EPACE-10	467.63 a	3.62 a	2.03 ab

Means followed by the same letters in the same column within each phase are not significantly different by SNK test at $P = 0.05$ probability level.

Table 3

Chlorophyll *a* fluorescence analysis, the maximum dark-adapted quantum yield efficiency of photosystem II (PSII) (Fv/Fm), and the effective quantum yield of PSII (Φ_{PSII}), under 500 and 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetic photon flux density, on four genotypes of cowpea in three reproductive stages.

Genotypes	Φ_{PSII} 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Φ_{PSII} 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Fv/Fm
PRE-FLOWERING STAGE			
Novaera	0.347 a	0.252 a	0.845 a
Gurguéia	0.402 a	0.173b	0.830b
Paulistinha	0.365 a	0.148c	0.835 ab
EPACE-10	0.364 a	0.186b	0.818c
FLOWERING STAGE			
Novaera	0.245 a	0.165 a	0.825 a
Gurguéia	0.266 a	0.122 a	0.814b
Paulistinha	0.309 a	0.149 a	0.820 ab
EPACE-10	0.307 a	0.160 a	0.819 ab
POD FILLING STAGE			
Novaera	0.289 a	0.149 a	0.824 a
Gurguéia	0.262 a	0.100 a	0.831 a
Paulistinha	0.260 a	0.100 a	0.834 a
EPACE-10	0.336 a	0.106 a	0.827 a

Means followed by the same letters in the same column within each phase are not significantly different by SNK test at $P = 0.05$ probability level.

Fv/Fm ratio of Paulistinha was not different from Gurguéia, while EPACE-10 showed the lowest value (Table 3). Novaera, Paulistinha, and EPACE-10 showed the higher Fv/Fm in the flowering stage, while Gurguéia had the lowest value. In the pod filling stage, there were no significant differences in these parameters among the genotypes.

3.3. Grain protein content

Among the genotypes, the TGPC ranged from 18.9 % for Gurguéia to 24.3 % for EPACE-10 (Table 4). The genotypes with higher TGPC were Paulistinha and EPACE-10 and were not significantly different, while the modern genotypes, Novaera and Gurguéia showed significantly lower and similar TGPC. Regarding the concentration of each protein fraction (Table 4), all four genotypes evaluated presented higher content of globulins, followed by the alkali glutelins, acid glutelins, albumins, and prolamins. Novaera, Paulistinha, and EPACE-10 showed significantly higher globulins contents than Gurguéia. Regarding albumins content, Paulistinha and EPACE-10 showed a significantly lower range than Novaera and Gurguéia, and Gurguéia showed less than Novaera (Table 4). The prolamins content of EPACE-10 was higher than the other genotypes, with significantly lower content for Novaera. The acid glutelins content of Novaera and Gurguéia was significantly lower than that for Paulistinha and EPACE-10, while EPACE-10 showed significantly higher content than the other three genotypes. Finally, Paulistinha and EPACE-10 showed significantly higher alkali glutelins than Novaera and Gurguéia, while Gurguéia showed more than Novaera (Table 4).

Table 4

Total grain protein contents and their fractions on four genotypes of cowpea.

Protein Fractions	Genotypes			
	Novaera	Gurguéia	Paulistinha	EPACE-10
Globulins (mg 100 mg^{-1})	15.5a	13.3b	15.6a	16.4a
Albumins (mg 100 mg^{-1})	0.6a	0.4b	0.4c	0.4c
Prolamins (mg 100 mg^{-1})	0.2c	0.3b	0.3b	0.4a
acid Glutelins (mg 100 mg^{-1})	0.9c	0.9c	1.0b	1.4a
alkali Glutelins (mg 100 mg^{-1})	2.8c	4.0b	5.7a	5.9a
Total grain protein (mg 100 mg^{-1})	20.0b	18.9b	23.0a	24.3a

Means followed by the same letters in the same line are not significantly different by SNK test at $P = 0.05$ probability level.

3.4. Grain amino acid content

Concerning amino acids (Table 5), the four genotypes presented high lysine content and low methionine and cysteine content (sulfur amino acids). The lysine content of Gurguéia and EPACE-10 was similar and higher than that of Novaera and Paulistinha. Novaera and Paulistinha showed similar methionine content; however, Gurguéia and EPACE-10 showed significantly lower content than Novaera, but not Paulistinha (Table 5). The cysteine content of all genotypes was very similar. All the genotypes showed high glutamic acid content, followed by aspartic acid, arginine, lysine, leucine, and proline contents. The other amino acids were below 1 g 100 g^{-1} DW.

3.5. Electrophoretic SDS-1D-PAGE profiles

The entire protein fraction presented a high dispersion of polypeptides varying molecular mass between 16 and 100 kDa (Fig. 1A). However, all four genotypes showed the same intensity of the bands for the globulins fraction (Fig. 1A). The 26 kDa band of albumins exhibited a higher intensity in all genotypes, but Paulistinha and EPACE-10 showed a higher intensity for all the bands than the other two genotypes (Fig. 1B). Regarding the prolamins fraction, Paulistinha and EPACE-10 also showed a higher intensity for all bands than the other two genotypes, and Gurguéia had less intense bands (Fig. 1C). SDS-PAGE for acid glutelins were similar among the genotypes but slightly more intense for EPACE-10 (Fig. 1D), which had shown high intensity for the band of 20 kDa polypeptide and from 70 to 100 kDa. The alkali glutelins fraction (Fig. 1E) showed a high intensity of all bands for Novaera, Paulistinha, and EPACE-10, but Gurguéia showed low intensity for all bands (Fig. 1E). In this study, polymorphism did not show significant differences for all protein fractions, only for the alkali glutelins fraction. Gurguéia showed a 35 kDa polypeptide, which was not evident for the other three genotypes (Fig. 1E).

3.6. Yield components

In this experiment, the number of pods per plant was not significantly different among the genotypes. Gurguéia showed a significantly higher number of grains per plant than the other three genotypes, which were similar to each other (Table 6). The grain weight per plant of Gurguéia and Novaera was identical and significantly higher than for EPACE-10 and Paulistinha, which were similar (Table 6).

4. Discussion

The evaluation of morphological parameters and LSPC revealed that Novaera had the higher leaf area in the vegetative stage. Still, in the flowering stage, the leaf area was higher for Paulistinha and EPACE-10 than for Novaera and Gurguéia, promoting an increase in the whole leaf CO_2 assimilation per plant in this stage when pho-

Table 5
Seed amino acid contents of four genotypes of cowpea.

Seed amino acids		Genotypes			
(g 100 g ⁻¹ DW)		Novaera	Gurguéia	Paulistinha	EPACE-10
Essentials	HIS	0.6c	0.6b	0.5c	0.7a
	VAL	0.9b	1.0b	0.9b	1.2a
	LYS	1.2b	1.4a	1.3b	1.4a
	ILE	0.7c	0.8b	0.7c	1.0a
	LEU	1.3c	1.4b	1.4b	1.71a
	PHE	0.9c	1.1b	1.0c	1.3a
	MET	0.6a	0.5b	0.6a	0.5b
	THR	0.8c	0.9b	0.8c	1.0a
	Non-essentials	CYS	0.1a	0.1a	0.1a
SER		0.9c	1.1b	0.9c	1.2a
GLU		3.5b	3.8b	3.7b	4.5a
GLY		0.8c	0.9b	0.7c	1.0a
ALA		0.8b	0.9b	0.8b	1.0a
ARG		1.4a	1.5a	1.3a	0.9a
PRO		1.0b	1.2b	1.0b	1.3a
TYR		0.8c	0.9b	0.8c	1.0a
ASP		1.7b	1.9b	1.9b	2.2a

HIS, histidine; VAL, valine; LYS, lysine; ILE, isoleucine; LEU, leucine; PHE, phenylalanine; MET, Methionine; THR, threonine; CYS, cysteine; SER, serine; GLU, glutamic acid; GLY, glutamine; ALA, alanine; ARG, arginine; PRO, proline; TYR, tyrosine; ASP, aspartic acid. Means followed by the same letters in the same line are not significantly different by SNK test at P = 0.05 probability level.

tosynthesis and leaf starch content is increased to sustain the future growth of the embryo as stated by Long et al. (2006) and Pimentel et al. (1999a). However, LSPC in this stage was the same for all the genotypes. Among LSPC, the enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), responsible for CO₂ assimilation in the Calvin cycle, accounts for more than 50 % of LSPC (Feller et al., 2007; Long et al., 2006). LSPC at the reproductive stages was correlated to yield in common bean (Barros et al., 2016), but this was not the case in this study with cowpea genotypes. Due to its abundance in leaves, the enzyme Rubisco is also responsible for the most substantial protein proportion degraded during reproductive stages to remobilize nitrogen for the grain (Feller et al., 2007). This phenomenon have been observed at the pod filling stage, denoting leaves' senescence and nitrogen remobilization to the grains (Pimentel, 2006). Paulistinha showed a significantly lower LSPC than the other genotypes at this stage. Probably this genotype had remobilized more nitrogen from the leaf to the grain to ensure its high TGPC (Gonçalves et al., 2020). Among the physiological parameters evaluated, only leaf area in the vegetative and flowering stage and LSCP in the pod filling stage showed some differences among the genotypes. Therefore, these differences were not enough to discriminate the genotypes apart from the differences of LSPC at the pod filling stage.

Analysis of the variables obtained by the emission of chlorophyll α fluorescence is a well-known rapid technique (Schreiber et al., 1994) used to evaluate the photosynthetic potential under different conditions (Murchie and Lawson, 2013). Photosynthesis is maximal at the pollination stage, i.e., the called pre-flowering stage, to ensure embryo growth after pollination, as shown before (Pimentel et al., 1999a). This phenomenon was observed in this experiment, by the high Φ_{PSII} and Fv/Fm values of all the genotypes, especially at this pre-flowering stage. The Φ_{PSII} values at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of PPFD were smaller than at 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for all genotypes at all reproductive stages, due to photoinhibition at high PPFD (Long et al., 2006). Novaera was the least sensitive to photoinhibition at the pre-flowering stage, with high Φ_{PSII} values and probably high CO₂ assimilation to ensure its higher yield. However, no physiological parameters used discriminated genotypes for yield and TGPC, as they did under stressful conditions (Pimentel et al., 1999b; Costa et al., 2002).

The TGPC varied from 18.9 to 24.3 % among the genotypes evaluated, demonstrating the high TGPC for cowpea (Rangel et al.,

2003; Vasconcelos et al., 2010). The genotypes Paulistinha and EPACE-10, used for low-input agriculture, showed very similar and significantly higher TGPC than Novaera and Gurguéia, used in high-input agriculture. The high content of globulins and albumins in cowpea grains is vital for human nutrition since these fractions are the richest in essential amino acids (Lookhart and Bean, 2000; Petsko & Ringe, 2004). The proportion of the protein fraction verified in this study (Table 4) is in agreement with those reported previously (Rangel et al., 2003; Gupta et al., 2010), except for the high content of alkali glutelins, probably due to the methodology used for acid and alkali glutelins extractions (Gonçalves et al., 2020). The elevated globulins content of the cowpea genotypes can be used to complement those of cereals for food safety (Elhardallo et al., 2015; FAO, 2017; Rangel et al., 2003). Cereals grains are rich in prolamins and glutelins (Lookhart and Bean, 2000), which are more deficient in the essential amino acids lysine but have a high methionine content (Petsko and Ringe, 2004). Cowpea is considered one of the high-quality plant protein sources in the tropics, especially in areas under environmental stresses (Elhardallo et al., 2015; Jayathilake et al., 2018; Rangel et al., 2003).

Concerning the grain amino acids content, EPACE-10 showed the highest content for all amino acids, except for methionine (a sulfur amino acid). The four cowpea genotypes presented high lysine and leucine contents and low methionine content, as described in the literature (Elhardallo et al., 2015; Vasconcelos et al., 2010). Most essential amino acids of cowpea, except methionine, were present at acceptable levels compared to the reference pattern for preschool children and adults (FAO/IFAD/UNICEF/WFP/WHO, 2020).

SDS-PAGE was successfully used to detect differences among the genotypes (Alghamdi et al., 2019; Jayathilake et al., 2018). The globulins fraction showed identical high intensity for the bands of all four genotypes (Fig. 1A). In contrast, the albumins and prolamin fractions profile showed differences among the genotypes, such that Paulistinha and EPACE-10 showed a higher intensity for all bands than for the others. The intensity of the bands for the albumin, prolamins, and glutelin fractions discriminated the genotypes better than the globulins fraction.

In this work, the yield of Novaera and Gurguéia was similar and significantly higher than for EPACE-10 and Paulistinha, which were identical to each other (Table 6). This study found a negative correlation between TGPC and yield, as stated in the literature

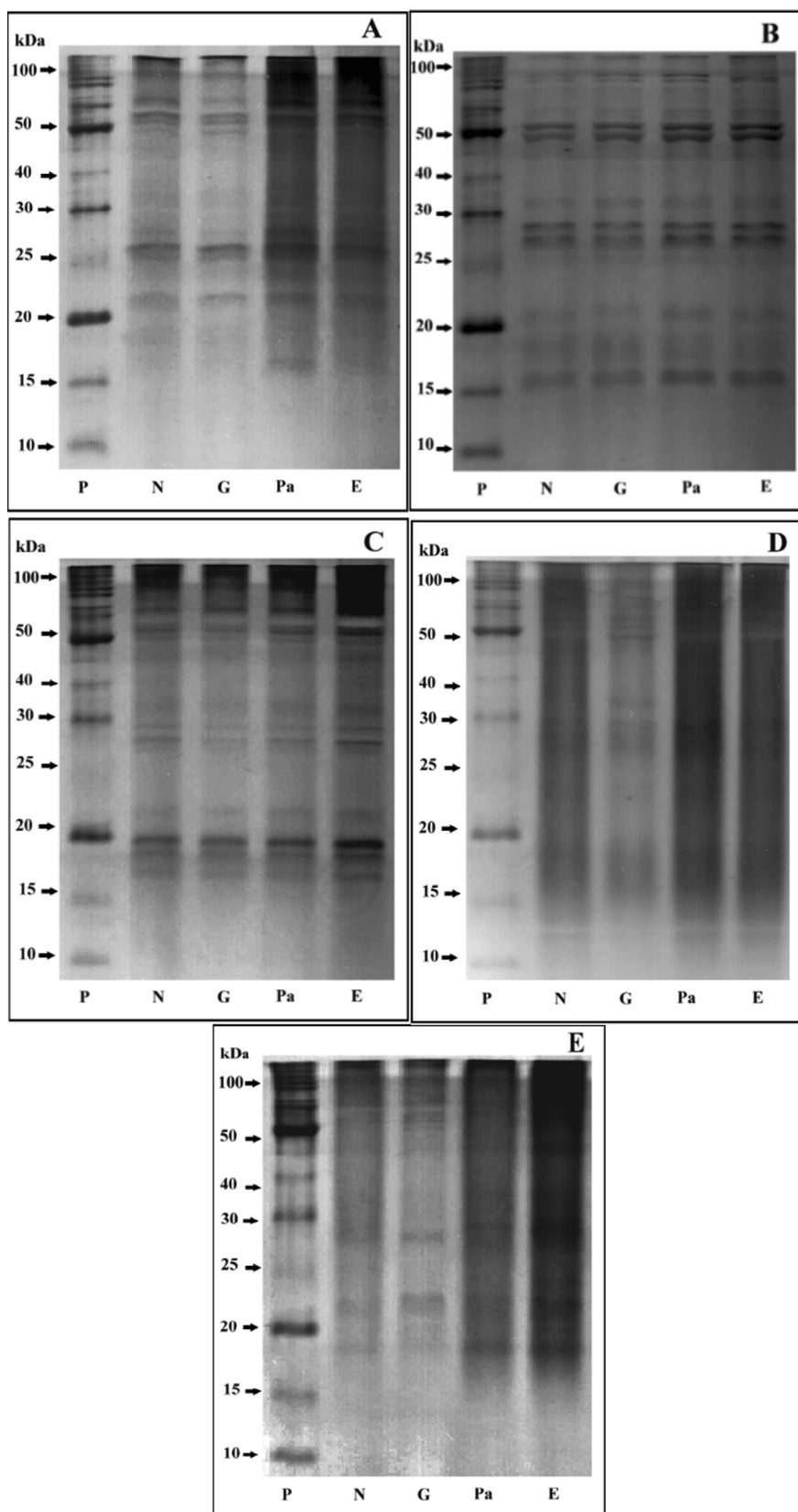


Fig. 1. Electrophoretic SDS-1D-PAGE profiles for globulins (A), albumins (B), prolamins (C), acid glutelins (D), and alkali glutelins (E) in seed storage protein of cowpea genotypes. Lane 1: Protein ladder, lane 2: Novaera, lane 3: Gurguéia, lane 4: Paulistinha, lane 5: EPACE 10.

for cereals (Rharrabti et al., 2001). The most productive genotypes, Novaera and Gurguéia, showed the lowest TGPC compared with the less productive local genotypes (Gonçalves et al., 2020)

but more adapted to environmental stresses (Gomez-Zavaglia et al., 2020), Paulistinha and EPACE-10. An increase in TGPC with no loss in grain biomass can only be accomplished when the

Table 6
Yield components of four genotypes of cowpea.

Genotype	Number of pods per plant	Number of grains per plant	Grain weight per plant (g)
Novaera	6.00 a	33.75b	8.622 a
Gurguéia	6.75 a	70.50 a	6.892 a
Paulistinha	3.75 a	28.00b	4.727b
EPACE-10	4.25 a	33.00b	3.800b

Means followed by the same letters in the same column are not significantly different by SNK test at P = 0.05 probability level.

photosynthetic capacity and nitrogen remobilization increase, as Long et al. (2006) and Feller et al. (2007) stated.

5. Conclusion

The evaluation of physiological variables related to photosynthesis and nitrogen content in the leaves was not very helpful in discriminating the yield and protein content of the genotypes under the optimal conditions of this essay, as they were under environmental stresses. Only the evaluation of the relationship between yield, TGPC, and amino acid content discriminated genotypes to produce low-cost proteins with quality. This study showed that. In marginal areas for agriculture, where population and food scarcity grow fast, the traditional local genotypes, such as Paulistinha and EPACE-10 studied here, can be indicated to produce more low-cost vegetal protein because of their high TGPC and environmental stresses adaptation. The high-yielding genotypes, Gurguéia and Novaera, are more sensitive to stresses and have a lower TGPC but higher yield. Therefore, they can be cultivated in high-input agriculture to produce more grains for food safety in the rest of the world. The traditional local cowpea genotype EPACE-10, adapted to environmental constraints, with high TGPC, globulin, and amino acid content than Paulistinha, can be recommended for cultivation in these marginal areas, where ambient stresses are frequent and will increase under future climate changes. Future studies on plant nutrition could help better understand the relationship between yield and grain protein content.

CRedit authorship contribution statement

Wedis Martins Ferreira: Data curation, Formal analysis, Methodology. **Gepatrik Rodrigues Lima:** Data curation, Methodology, Validation. **David Cabral Macedo:** Data curation, Methodology, Validation. **Murillo Freire Júnior:** Methodology, Validation, Writing – review & editing. **Carlos Pimentel:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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