

# Characterization of the development of cowpea cultivars and of the quantity and quality of proteins in their grains

**Abstract** – The objective of this work was to evaluate cowpea (*Vigna unguiculata*) cultivars regarding plant development and the quantity and quality of soluble proteins in their grains, for breeding purposes. The experiment was conducted in a greenhouse, in a completely randomized experimental design, with the Paulistinha, BRS Novaera, Epace 10, and BR 17-Gurguéia cultivars. Leaf area, shoot fresh and dry matter, leaf protein content, number of nodules, and nodule and root dry matter were evaluated. In mature grains, soluble fractions and soluble amino acids were also quantified, and the electrophoretic analysis was performed with protein denaturation. There were no differences between cultivars for the plant development variables. However, protein quantity and quality in the grain differed between cultivars. 'BRS Novaera' and 'BR 17-Gurguéia' showed a higher soluble protein content in their grains. 'BRS Novaera' exhibited higher contents of two soluble sulfur amino acids – methionine and cysteine – , not differing from 'BR 17-Gurguéia' regarding methionine content. Both cultivars presented protein band polymorphism, but BRS Novaera had an extra band for albumins. The BRS Novaera and BR 17-Gurguéia cultivars have the highest content of soluble proteins in their grains and the greatest protein polymorphism, which makes them suitable for improving the nutritional quality of cowpea.

**Index terms:** *Vigna unguiculata*, amino acids, grain protein, SDS-PAGE.

## Caracterização do desenvolvimento de cultivares de feijão-caupi e do conteúdo e da qualidade proteica dos seus grãos

**Resumo** – O objetivo deste trabalho foi avaliar cultivares de feijão-caupi (*Vigna unguiculata*) quanto ao desenvolvimento da planta e à quantidade e à qualidade de proteínas solúveis em seus grãos, com vistas ao melhoramento. O experimento foi conduzido em casa de vegetação, em delineamento experimental inteiramente casualizado, com as cultivares Paulistinha, BRS Novaera, Epace 10 e BR 17-Gurguéia. Avaliaram-se área foliar, peso fresco e seco da parte aérea, conteúdo de proteína foliar, número de nódulos, e massa seca de nódulos e de raiz. Nos grãos maduros, também quantificaram-se as frações solúveis e os aminoácidos solúveis, e a análise eletroforética foi realizada com desnaturação das proteínas. Não houve diferenças entre as cultivares quanto às variáveis do desenvolvimento da planta. No entanto, a quantidade e a qualidade das proteínas nos grãos diferiram entre as cultivares. 'BRS Novaera' e 'BR 17-Gurguéia' apresentaram maior teor de proteína solúvel nos seus grãos. 'BRS Novaera' apresentou maior teor de dois aminoácidos sulfurados solúveis – metionina e cisteína – , não tendo diferido de 'BR

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17-Gurguéia' quanto ao conteúdo de metionina. Ambas as cultivares apresentaram polimorfismo de bandas, mas BRS Novaera apresentou uma banda extra para albuminas. As cultivares BRS Novaera e BR 17-Gurguéia apresentam o maior conteúdo de proteínas solúveis nos seus grãos e o maior polimorfismo de proteínas, o que as torna adequadas para o melhoramento nutricional do feijão-caupi.

**Termos para indexação:** *Vigna unguiculata*, amino ácidos, proteína do grão, SDS-PAGE.

## Introduction

Pulses are second in agricultural importance, only behind Gramineae (Singh et al., 2007). They provide a good source of protein, ranging from 18 to 35%, and supplement cereals with protein, minerals, and vitamins of the B complex (Elhardallou et al., 2015). Among the grain legumes, cowpea [*Vigna unguiculata* (L.) Walp.] is considered an important source of protein, essential minerals (Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn, for example), vitamins (A, C, B1, B2, B5, B6, and B9), carbohydrates, and antioxidants that are fundamental for human health, growth, and development (Gerrano et al., 2019). In Brazil, the crop is cultivated throughout the entire country, especially in the Northern and Northeastern regions and, more recently, in the Midwest.

The genetic variability among cowpea genotypes for different proteins helps to improve the nutritional quality of the species through genetic breeding (Gerrano et al., 2019). The 'BRS Novaera', 'BR 17-Gurguéia', 'Paulistinha', and 'Epace 10' genotypes, for example, were developed to increase the production potential, resistance to viruses, tolerance to drought, and commercial quality of grains and pods. However, although agronomic data about these genotypes is available, more detailed information on grain nutritional characteristics is still necessary.

Further information on cultivar characterization, including shoot development, nodulation, and leaf soluble protein content, may help in the search for answers regarding nitrogen assimilation and remobilization for the synthesis of grain proteins. Leaf proteins and, in particular, photosynthetic proteins of plastids are extensively degraded during senescence, providing a large source of N that plants can tap to supplement the nutrition of growing organs such as

new leaves and seeds (Masclaux-Daubresse et al., 2008).

Biological N fixation (BNF) is a key source of N for cowpea, which is one of the crops with the highest protein content and, therefore, a high demand for the nutrient. Studies carried out with soybean [*Glycine max* (L.) Merr.] and common bean (*Phaseolus vulgaris* L.) have already shown that N derived from BNF is more easily moved to pods during grain filling than nitrate-N (Santos et al., 2013). In addition, characteristics related to the nodulation process, such as number of nodules, nodule dry matter, and root dry matter, may indicate nodulation efficiency, mainly when evaluated at important phenological stages such as flowering.

Therefore, data on nutritional and physiological parameters is vital to help plant breeders choose high-protein lines (Ravelombola et al., 2016). Due to the great diversity in their protein content, different cowpea cultivars have been characterized in recent studies, aiming for further application in breeding programs with a considerable interest in improving the nutritional quality – in terms of essential amino acids – of high-protein legumes (Gupta et al., 2010).

The objective of this work was to evaluate cowpea cultivars regarding plant development and the quantity and quality of soluble proteins in their grains, for breeding purposes.

## Materials and Methods

The study was carried out in a greenhouse belonging to the Department of Crop Science of Universidade Federal Rural do Rio de Janeiro, located in the state of Rio de Janeiro, Brazil (22°45'S, 43°41'W, at an altitude of 40 m). The predominant climate in the region is Aw according to Köppen-Geiger's classification, with hot and rainy summers and dry winters. Plants were cultivated in 8.0-L pots containing an Argissolo Vermelho-Amarelo, i.e., a Kanhapudalf, with the following chemical characteristics: 2.3 mg dm<sup>-3</sup> P, 72 mg dm<sup>-3</sup> K (KCl 1.0 mol L<sup>-1</sup>), 1.8 cmol<sub>c</sub> dm<sup>-3</sup> Ca<sup>+2</sup>, 0.8 cmol<sub>c</sub> dm<sup>-3</sup> Mg<sup>+2</sup>, and 0 cmol<sub>c</sub> dm<sup>-3</sup> Al<sup>+3</sup> (HCl 0.05 mol L<sup>-1</sup> + H<sub>2</sub>SO<sub>4</sub> 0.0125 mol L<sup>-1</sup>). Fertilization was carried out according to Manual de Calagem e Adubação do Estado do Rio de Janeiro (Freire, 2013), except for N, which was not supplied to the plants, following the usual method of cultivation. Planting was carried out on

5/16/2016, and the grains were inoculated with Semia 6464 (BR 3262), a specific strain of *Bradyrhizobium* sp., obtained from the germplasm bank of Embrapa Agrobiologia and currently recommended to supply N to cowpea. Inoculation was performed as described in Hungria et al. (2003), aiming to allow biological fixation without growth promotion, which is the current practice.

The experimental design was completely randomized, with four treatments (four cultivars) and eight replicates; each experimental plot consisted of two plants. The cowpea cultivars studied were Epace 10, BR 17-Gurguéia, Paulistinha, and BRS Novaera. In order to better characterize the behavior of these genotypes, they were analyzed in four different periods, which were: vegetative growth, from planting to the appearance of the first floral bud; pre flowering, when more than 50% of the plants had at least one bud – at this stage, the root and its nodulation were assessed; full flowering, when at least 50% of the plants had a fully-expanded flower; pod filling, which begins with the filling of the first pod; and grain storage, when proteins and soluble amino acids were evaluated. The sampling time in days after planting of each cowpea cultivar is detailed in Table 1.

Soluble leaf protein was quantified according to Bradford (1976). The leaf fraction analyzed corresponded to the central leaflet of the third youngest and fully-expanded leaf.

The shoot was separated from the cut roots at the cotyledon insertion point near the stem base, and leaves were separated to measure leaf area using the LI-3000C portable leaf area meter (LI-COR, Lincoln, NE, USA). Nodules were separated from roots, counted, and dried at 60°C for 72 hours, and then used to evaluate the potential of BNF. The number of nodules was used to indirectly reveal the relationship between

fixation potential and quantitative characteristics (Ferreira et al., 2011).

The grains were harvested at physiological maturation. For the extraction of storage proteins, dried grains were lyophilized and ground, following the method described by Vasconcelos et al. (2010) with some adaptations. Samples of 100 mg each, in three replicates, were taken from a single sample of flour from the grains of different plants. The samples were treated with 1.0 mL hexane, at room temperature, for 15 min, slightly shaken, centrifuged at 12.000 g for 5 min, and allowed to dry overnight. The extraction process was sequential, allowing the precipitate from the previous extraction to be used as a pellet for the next extraction. The extraction of the sequential protein fraction included: three globulin extractions with 1.0 mL NaCl (0.5 mol L<sup>-1</sup>); three albumin extractions with 1.0 mL distilled water, with the first extraction being discarded; one prolamin extraction with 0.5 mL ethanol (70%); and two glutelin extractions, i.e., acidic glutelin extracted with 1.0 mL HCl (0.1 mol L<sup>-1</sup>) and basic glutelin extracted with 1.0 mL NaOH (0.1 mol L<sup>-1</sup>), in this order.

The centrifugations for the extraction of prolamins and glutelins were performed at 25°C, i.e., at room temperature, while the extractions of albumins and globulins were carried out at 4°C. The protein supernatant was retrieved after centrifugation, and, when two centrifugations were performed, both supernatants were combined and stored at -80°C.

The protein fraction content of albumins, globulins, prolamins, and acid glutelins were determined according to Bradford (1976), whereas, the method used for basic glutelin was similar to that of Lowry et al. (1951). All samples were quantified using the Protein Assay and DC Protein Assay kits (Bio-Rad Laboratories, Inc., Hercules, CA, USA), and bovine

**Table 1.** Sampling time in days after planting (DAP) of the four cowpea (*Vigna unguiculata*) cultivars in four different periods.

Cultivar	Sampling time in days after planting			
	Vegetative growth	Pre flowering	Full flowering	Pod filling
BRS Novaera	21	49	52	60
BR 17-Gurguéia	21	49	60	68
Paulistinha	21	49	60	68
Epace 10	21	49	60	68

albumin serum was used as a standard. The values were obtained in relation to a standard curve by linear regression. The total percentage of the soluble protein was based on the sum of the protein fractions.

The electrophoretic analysis was carried out under denaturing conditions of 0.1% (w/v) sodium dodecyl sulfate in 13% polyacrylamide gel, with known concentrations of protein loaded onto each lane for each storage protein fraction. The general running conditions were determined at a constant current of 15 mA per gel. Molecular mass markers were applied to each gel to determine the molecular mass of the proteins, and the gels were prepared and stained with a solution of silver nitrate according to Morrissey (1981).

Soluble amino acid extraction was performed as in Bieleski & Turner (1966). A 1.0-g sample of lyophilized flour was mixed into a 10-mL solution of methanol, chloroform, and water, in the 12:5:3 ratio, and was incubated overnight at 4°C. The samples were centrifuged at 10.000 g for 20 min, at 4°C, to remove cell debris, and the supernatant was collected and mixed with 1.0 mL chloroform and 1.5 mL ultrapure water. The two layers formed were separated by centrifugation at 10.000 g for 20 min at 4°C. The aqueous stage was carefully removed and incubated at 38°C in a water bath for 1 hour and then lyophilized. The pellet was resuspended in 1.0 mL distilled-deionized water, and the solution of soluble amino acids was frozen at -20°C before the separation and quantification of the amino acids.

Lyophilized samples were reconstituted in ultrapure water. A volume of 10 µL was derivatized according to the manufacturer's recommendations in 70 µL borate buffer and 20 µL of the AccQ-fluor derivative agent (Waters Corporation, Milford, MA, USA). The mixture was heated to 55°C for 10 min and, after it cooled down, 1.0 µL of each sample was used for separation and quantification through the Acquity UPLC system (Waters Corporation, Milford, MA, USA). In the UPLC system, the reverse phase separation was performed with the BEH C18 column (100 mm × 2.1 mm i.d., 1.7 µm) at 46°C, with a flow rate of 0.7 mL per min between the following eluents: AccQ-Tag Waters, 10% acetonitrile, 100% Milli-Q water, and 100% acetonitrile. The derivatized product was detected at 260 nm. The amino acids histidine, serine, arginine, glycine, aspartate, threonine, alanine, proline, cysteine, lysine, tyrosine, methionine, valine,

isoleucine, leucine, and phenylalanine were determined based on the amino acid standard H, product number NCI0180 (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical differences in the quantitative characteristics among genotypes were compared by the LSD test, at 5% probability, using the Sisvar software (Universidade Federal de Lavras, Lavras, MG, Brazil).

## Results and Discussion

The BRS Novaera cultivar showed the highest values for shoot fresh and dry matter and leaf area at the vegetative growth stage (Table 2). The leaf protein content was significantly higher in the Paulistinha cultivar, compared with BRS Novaera and BR 17-Gurguéia.

At pre flowering, cultivars differed regarding leaf protein and root dry matter (Table 3). 'BRS Novaera' and 'Epace 10' exhibited the lowest and highest leaf protein contents, respectively, while 'Paulistinha' had a greater root dry matter. The variables number of nodules and nodule dry matter did not differ significantly among cultivars, varying from 127 to 182 and from 0.36 to 0.49, respectively (Table 3). Since the number of nodules was close to that reported in the literature, all cultivars received an adequate supply of N by FBN, similarly to what was observed by Ferreira et al. (2011). Silva et al. (2012), for example, studying the application of different inoculum rates in the BRS Pujante cowpea cultivar, found a greater accumulation of N in the shoot of inoculated plants than in that of those fertilized with N, besides less than 80 nodules per plant.

At full flowering, when 50% of the plants had the first fully-open flower, significant differences were observed for shoot fresh and dry matter and leaf protein (Table 2). At this stage, the BR 17-Gurguéia cultivar showed the highest values for shoot dry matter and leaf protein.

In general, leaf protein content increased until the pre-flowering stage, decreasing afterwards. This behavior was evident mainly for the BR 17-Gurguéia cultivar (Table 2), which, at full flowering, presented a leaf protein content considerably higher than that of the other cultivars; however, at the stage of pod filling, the cultivars did not differ regarding this characteristic. The observed decrease in protein content may be

associated with leaf protein mobilization in function of the soluble protein content in the grains, since 'BR 17-Gurguéia' showed a high total soluble protein. Therefore, grain protein content was inversely proportional to leaf protein content; for instance, the Epacé 10 cultivar, which remained with a higher

leaf protein content until the pod-filling stage, also showed a lower grain soluble protein content than BR 17-Gurguéia. According to Peoples et al. (1983), each fruit absorbs N from all available sources, but leaf N is preferentially distributed to nearby fruits as the lower fruits monopolize the N exported from the nodulated

**Table 2.** Averages of leaf area, shoot fresh matter (SFM), shoot dry matter (SDM), and leaf protein content of four cowpea (*Vigna unguiculata*) cultivars at four phenological stages<sup>(1)</sup>.

Cultivar	Leaf area (cm <sup>2</sup> )	SFM (g)	SDM (g)	Protein (mg BSA g <sup>-1</sup> FM) <sup>(2)</sup>
Vegetative growth				
BRS Novaera	364.5a	14.15a	1.27a	2.23b
BR 17-Gurguéia	263.6ab	8.74b	0.83b	2.33b
Paulistinha	254.7b	8.95b	0.87b	2.58a
Epacé 10	289.4ab	10.22ab	0.95ab	2.34ab
CV (%)	18.91	20.88	18.98	8.64
Pre flowering				
BRS Novaera	1,040.6a	46.645a	8.52a	3.40b
BR 17-Gurguéia	1,099.9a	42.990a	6.99a	3.65b
Paulistinha	1,199.9a	44.752a	7.37a	3.52ab
Epacé 10	1,027.1a	42.133a	6.48a	3.67a
CV (%)	17.54	18.49	21.62	5.68
Full flowering				
BRS Novaera	1264.3a	47.44b	10.39b	3.00b
BR 17-Gurguéia	1638.7a	75.01a	16.10a	3.85a
Paulistinha	1237.3a	53.28b	9.31b	2.99b
Epacé 10	1413.5a	61.33ab	11.10b	2.96b
CV (%)	16.44	6.46	19.5	5.39
Pod filling				
BRS Novaera	1173.3a	84.05a	16.43a	2.49ab
BR 17-Gurguéia	1286.9a	81.03a	16.73a	2.67ab
Paulistinha	1329.7a	68.16a	13.49a	2.40b
Epacé 10	1297.6a	66.78a	12.68a	2.72a
CV (%)	37.33	21.06	17.83	9.44

<sup>(1)</sup>Means followed by equal letters, in the columns, within each phase, do not differ by the LSD test, at 5% probability. <sup>(2)</sup>BSA, bovine albumin serum; and FM, fresh mass.

**Table 3.** Number of nodules (NN), nodule dry matter (NDM), and root dry matter (RDM) of four cowpea (*Vigna unguiculata*) cultivars at the pre-flowering stage<sup>(1)</sup>.

Cultivar	Pre flowering		
	NN	NDM	RDM
BRS Novaera	182a	0.46a	2.21b
BR 17-Gurguéia	127a	0.36a	2.04b
Paulistinha	174a	0.49a	4.00a
Epacé 10	166a	0.39a	2.41b
CV (%)	27.05	29.69	29.01

<sup>(1)</sup>Means followed by equal letters, in the columns, do not differ by the LSD test, at 5% probability.

roots during late fructification. The mobilization of N fixed before flowering contributed with 60% of the fruits' total intake of the nutrient, while the N fixed after flowering contributed with the remaining 40% (Peoples et al., 1983).

The obtained results showed a great similarity among cultivars for leaf area, shoot fresh and dry matter, leaf protein content, and root dry matter. Therefore, since only punctual differences were observed, it was not possible to establish a pattern for the response of the cultivars to the environment. However, Sinclair & Vadez (2012) and Ferreira et al. (2011) found a close correlation between N storage in the vegetative tissue and crop mass. According to Sinclair & Vadez (2012), N can be stored during vegetative development only when there are tissues available to receive the fixed N; therefore, the amount of N a plant can store in the vegetative tissue before seed growth is crucial to determine the total amounts of fixed N. Ferreira et al. (2011) reported a significant correlation between grain dry matter and the parameters number of nodules, plant dry matter, and N accumulated by the plants.

Regarding protein content, the two main fractions, globulin and albumin, represented about 84 and 2% of the total storage proteins, respectively (Table 4). The studied cultivars did not differ as to globulin content. For albumin, two cultivars were contrasting, Paulistinha, with 0.55%, and Epace 10, with 0.66%. According to Sharma & Krishna (2017), legume grains, in general, predominantly contain 20–35% albumins and 43–55% globulins, which, together, account for 63–90% of the total grain proteins. In the present study, the albumin content was lower than those found by Sharma & Krishna (2017) and also

than the mean of 27.20% for albumins and 48.76% for globulins obtained by Tchiagam et al. (2011).

The reduced albumin content observed in the present work is possibly related to the extraction method used. Although albumin is, by definition, water soluble proteins, most studies with cowpea (Odeigah & Osanyinpeju, 1996; Vasconcelos et al., 2010; Sharma & Krishna, 2017) used a saline solution to extract a mixture of globulins and albumins, which were subsequently separated by dialysis. In the method used in this study, the globulin fraction was extracted with 0.5 mol L<sup>-1</sup> NaCl, while the albumin fraction was extracted, in a following step, with distilled water, which, in comparative terms, can cause globulin overestimation and albumin underestimation.

Globulins are nutritionally deficient in cysteine and methionine, whereas albumins are rich in lysine, cysteine, and methionine (Clemente et al., 1998). For this reason, albumins are often considered a better amino acid and balanced group of proteins than globulins (Clemente et al., 1998). Therefore, the albumin content in the grains may be an interesting criterion for the selection of cultivars in breeding programs that aim to increase the nutritional quality of the grain, especially when there is no significant difference in globulin contents, as noted in the present study.

The contents of prolamins and of acid and basic glutelins were also significant. The highest values were found for 'BRS Novaera', with 0.5 for prolamins and 4.31 and 0.86 g 100 g<sup>-1</sup> for basic and acid glutelins, respectively (Table 5). The total soluble protein content ranged from 28.63 to 33.38% for 'Epace 10' and 'Paulistinha', respectively.

**Table 4.** Storage protein fractions, expressed as percentage of dry flour, in the grains of four cowpea (*Vigna unguiculata*) cultivars<sup>(1)</sup>.

Cultivar	Protein fraction (g 100 g <sup>-1</sup> )					Total soluble protein
	Albumin	Globulin	Prolamin	Basic glutelin	Acid glutelin	
BRS Novaera	0.61ab	24.61a	0.5a	4.31a	0.86a	30.88ab
BR 17-Gurguéia	0.63ab	26.77a	0.35c	3.10c	0.77b	31.62ab
Paulistinha	0.55b	27.88a	0.43b	3.68b	0.84a	33.38a
Epace 10	0.65a	25.21a	0.37bc	1.83d	0.57c	28.63b
CV (%)	10.25	10.25	9.27	6.92	6	8.7

<sup>(1)</sup>Means followed by equal letters, in the columns, do not differ by the LSD test, at 5% probability.

The fractions albumin, prolamin, and basic and acidic glutelins showed significant differences, with contents similar to those reported by Gupta et al. (2010), who found prolamin contents from 0.64 to 1.4 g 100 g<sup>-1</sup> and total basic and acid glutelin contents from 4.27 to 4.01 g 100 g<sup>-1</sup>. The prolamin content presents a negative correlation with the lysine content, i.e., cultivars with a high content of prolamins tend to have a low lysine content, which, in nutritional terms, is not good because lysine is a key essential amino acid that, together with threonine, methionine and isoleucine, is derived from the aspartic acid metabolic pathway (Azevedo et al., 2006; Gupta et al., 2010).

The protein band profiles of all four cultivars varied in number, width, and intensity (Figure 1). The relative molecular mass ranged between 10 and 90 kDa for the albumin fraction, 15 and 100 kDa for globulins, 15 and 90 kDa for prolamins, and 15 and 100 kDa for acid and basic glutelins. BRS Novaera exhibited different band patterns for albumins, globulins, and basic glutelins, compared with the other cultivars. It also presented one polypeptide between 10 and 15 kDa for albumin,

a lower intensity band between 20 and 25 kDa for the globulin fraction, and the suppression or non-detection of the band of 40 kDa for acid glutelin. For the BR 17-Gurguéia cultivar, the prolamin bands between 50 and 70 kDa were absent or not detected.

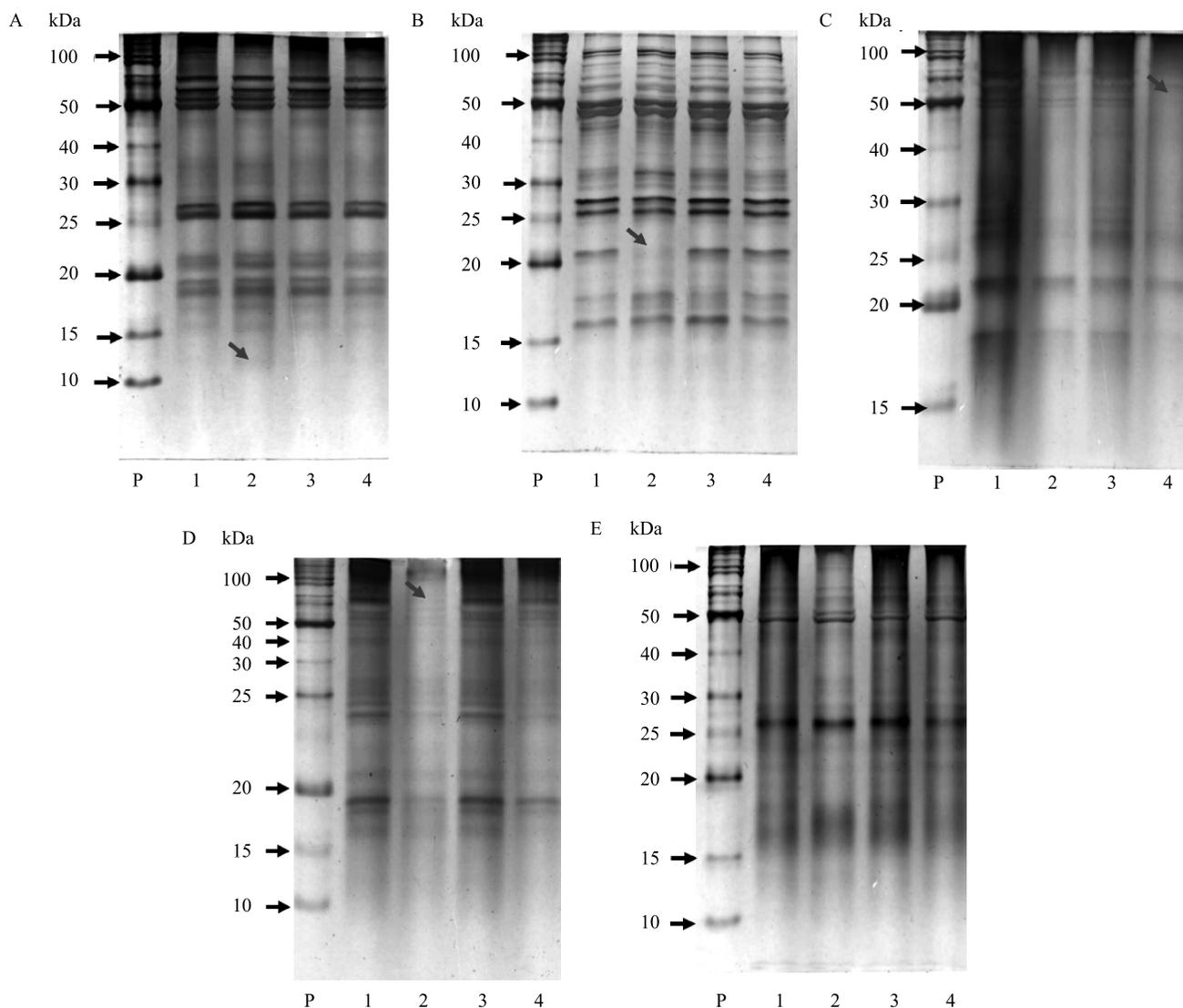
The polymorphism shown by the BRS Novaera and BR 17-Gurguéia cultivars can be better exploited in assisted selection studies or in studies that seek to identify allergy-causing proteins in legumes, since several classes of storage proteins have been associated with these types of allergies. Most of the bands were common in all cultivars, which indicates a close relationship among them (Figure 1). Gupta et al. (2014) found bands ranging from 15.85 to 147.9, 10 to 125.9, 7.94 to 56.23, and 10 to 79.43 kDa for the albumin, globulin, prolamin, and glutelin contents, respectively, of 11 cowpea genotypes (CS88, Chirrodi, HC98-64, HC6, LST-IIC12, CP16, CP21, COVU702, HC5, V240, and FS68).

Only seven amino acids (histidine, arginine, cysteine, lysine, tyrosine, methionine, and phenylalanine) differed significantly among cultivars

**Table 5.** Soluble amino acid content in the grains of four cowpea (*Vigna unguiculata*) cultivars<sup>(1)</sup>.

Soluble amino acid	Cultivar				CV (%)
	BRS Novaera	BR 17-Gurguéia	Paulistinha	Epace 10	
Essential (µg amino acid per 100 g dry flour)					
Histidine	0.422b	0.729ab	0.995a	0.813ab	25.06
Threonine	0.118b	0.107b	0.105b	0.105b	15.47
Lysine	0.048a	0.045ab	0.047a	0.041b	28.44
Methionine	0.029a	0.025ab	0.024b	0.023b	24.28
Phenylalanine	0.440b	0.386b	0.495b	0.916a	5.11
Isoleucine	0.053b	0.053b	0.061b	0.058b	10.31
Leucine	0.047b	0.037b	0.044b	0.047b	8.64
Nonessential (µg amino acid per 100 g dry flour)					
Arginine	0.599bc	0.308c	1.768a	0.804b	14.68
Aspartate	0.245a	0.410a	0.468a	0.366a	19.27
Serine	0.092a	0.089a	0.198a	0.151a	17.65
Alanine	0.206a	0.192a	0.199a	0.147a	17.54
Cysteine	0.020a	0.013b	0.014b	0.014b	33.7
Tyrosine	0.206b	0.083c	0.217ab	0.256a	25.91
Valine	0.127a	0.131a	0.182a	0.182a	24.6
Proline	0.238a	0.228a	0.308a	0.171a	24.56
Glycine	0.107a	0.084a	0.102a	0.083a	27.33

<sup>(1)</sup>Means followed by equal letters, in the columns, do not differ by the LSD test, at 5% probability.



**Figure 1.** SDS-ID electrophoretic profile of cowpea (*Vigna unguiculata*) cultivars, for: A, albumins; B, globulins; C, prolamins; D, acid glutelin; and E, basic glutelin. Lanes: P, molecular mass standard; 1, Paulistinha cultivar; 2, BRS Novaera cultivar; 3, Epace 10 cultivar; and 4, BR 17-Gurguéia cultivar. The arrows indicate band positions: A, presence of an extra band; B, lower band intensity; and C and D, band suppression.

(Table 5). BRS Novaera showed the highest contents of sulfur amino acids (methionine + cysteine -  $0.049 \mu\text{g } 100 \text{ g}^{-1}$ ) and did not differ from the other cultivars as to globulin content. This result differs from that of Gupta et al. (2010), who found a negative correlation of both globulins and sulfur amino acids (methionine and cysteine) with prolamins and lysine. In the present study, there was no correlation between prolamin and

lysine contents, since both 'Paulistinha', with more prolamin, and 'BR 17-Gurguéia', with less prolamin, did not differ as to lysine.

## Conclusions

1. The BRS Novaera and BR 17-Gurguéia cowpea (*Vigna unguiculata*) cultivars have the highest content

of soluble sulfur amino acids and methionine in their grains, with the presence of polymorphic bands, which makes them an important genetic material for breeding programs, aiming to improve the nutritional quality of the species.

2. Protein quantity and quality variables in the grain differ greatly between cultivars, showing a greater applicability in selection programs.

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