An Acad Bras Cienc (2020) 92(suppl.1): e20180484 DOI 10.1590/0001-3765202020180484
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

AGRARIAN SCIENCES

Protein, Phytate and Minerals in Grains of Commercial Cowpea Genotypes

FABÍOLA V. GONÇALVES, LEONARDO O. MEDICI, MARCOS PAULO S. DA FONSECA, CARLOS PIMENTEL, SALETE A. GAZIOLA & RICARDO A. AZEVEDO

Abstract: The objective of this study was to investigate and characterize cowpea (*Vigna unguiculata*) genotypes for total grain protein content, storage protein fractions (globulin, albumin, prolamin, basic and acid glutelins), and phytate and minerals contents. Eighteen cowpea genotypes were selected. Total grain protein content varied from 21.4% to 29.2%, for BRS Marataoã and Paulistinha genotypes, respectively. The variation in the concentration of each protein fraction was significant ($P<0.05$) only for glutelins (basic and acid). The genotypes studied exhibited great similarity in the PAGE electrophoretic profile of the grain protein fractions and also in the mineral content. BRS Paraguaçu genotype exhibited higher Zn content than thegenotypes that have been previously recommended for this characteristic. The lowest phytate grain content was observed in four of the 18 genotypes studied, which also exhibited high protein contents. Although the results did not converge to the selection of a few genotypes, some specific differences were detected that which may be further explored. Considering total grain protein, mineral and phytate contents, the genotype Paulistinha revealed a better balance unveiling high grain total protein content, low grain phytate content and more homogeneous mineral composition.

Key words: SDS-PAGE, minerals, phytate, total protein, *Vigna unguiculata*.

INTRODUCTION

About 20 leguminous species are used as dry grains for human nutrition because they are considered good sources of proteins, carbohydrates, water-soluble vitamins and minerals (Sreerama et al. 2012, Klupšaitė & Juodeikienė 2015). Pulses are the main source of dietary protein for a large part of the population, mainly in Asia, Africa and South America (Jafari et al. 2016). More recently, in addition to this important role, there is increasing evidence showing that there are many added health benefits when consuming pulses. For example, populations with high intakes of pulses have lower risk of diabetes, cardiovascular disease and obesity (Jafari et al. 2016).

Among the pulses species that stand out due to their high grain protein content is cowpea (*Vigna unguiculata* (L.) Walp.). Cowpea is an important grain legume cultivated in many parts of the world, in most tropical regions, especially in West Africa (Coulibaly & Lowenberg-DeBoer 2002, Abaidoo et al. 2017). Cowpea has its origin in the southern African region and it is now cultivated in more than 100 countries between 40ºN and 30ºS latitudes (Gonçalves et al. 2016).

In general, the nutritional profile of cowpea grains is similar to other legumes, with a relatively low-fat content and total protein content that is two to four-fold higher than that

of cereals and tubers (Timko & Singh 2008). The storage proteins of the grain are rich in the amino acids lysine (Lys) and tryptophan (Try), which are essential for monogastric animals (Azevedo et al. 1997, 2006), when compared to those of cereal grains, but poor in methionine (Met) and cysteine (Cys) when compared to proteins from animal sources (Timko & Singh 2008).

Cowpea grain is also an excellent source of minerals, especially iron (Fe) and zinc (Zn) (Pereira et al. 2014). Muranaka et al. (2016) reported a positive correlation for cowpea genotypes between total grain protein and Fe and Zn contents, which can be used in plant breeding research allowing the improvement of the total protein, Fe and Zn contents, without adverse interactions.

The use of cowpea grain as a high-quality protein food and the enrichment of farinaceous and functional foods from cowpea proteins has been limited by the low digestibility of the grains, deficiency in sulfur amino acids and the presence of antinutritional factors such as inhibitors of trypsin, oligosaccharides and phenolic compounds (Sreerama et al. 2012, Elhardallou et al. 2015).

Phytate (or phytic acid) is considered an antinutritional factor by forming complexes with essential minerals such as Ca, Zn and Fe. These complexes are very stable even at low pH (3 or 4) and are not readily digested within the gut. Consequently, the utilization of phosphorus (P) in the form of phytate (main form of storage of P in many plant tissues) is poor in non-ruminant animals as they do not possess endogenous phytases (Clarke & Wiseman 2000, Sandberg 2002, Kumar et al. 2010). Phytate has also been reported to form phytate-protein complexes, modifying protein structure (Coelho et al. 2002, 2005), which may result in reduction in solubility, enzymatic activity and proteolytic digestibility (Clarke & Wiseman 2000, Gonçalves et al. 2016). Nevertheless, several authors have reported some potential positive effects of phytate such as anticancer properties, antioxidant by complexing Fe and thereby reducing free radical generation and the peroxidation of membranes, and may also act in the regulation of insulin secretion (Coelho et al. 2002, Kumar et al. 2010, Gonçalves et al. 2016). These properties, considered to be promoters of general health improvements and disease prevention, have attracted the interest of both researchers and food manufacturers (Jacobs & Steffen 2003). Phytate is normally abundant in legume grains, including cowpea, and its concentration has been shown to vary from 2.6 to 15.2 g kg^{-1} (Gonçalves et al. 2016).

The total protein content of the cowpea grain has high heritability and is controlled by few genes, which allows the development of new cowpea genotypes with high protein content (Ravelombola et al. 2016). Studies indicate that the relative content of the protein fractions and the mineral composition of cowpea grains present great variation as a function of the cultivar (Kachare et al. 1988, Gonçalves et al. 2016). Due to the large number of genotypes available, the importance of this crop as a protein source and the growing scientific interest in the grain chemical composition (antinutritional and mineral factors), this study aimed to investigate and to characterize the grains of 18 cowpea genotypes for total protein content, storage protein fractions (globulin, albumin, prolamin, basic glutelin and acid glutelin), phytate and minerals contents.

MATERIALS AND METHODS

Plant material

Eighteen commercial cowpea genotypes with characteristics such as high productivity and high total grain protein content, and those commonly recommended for cultivation in the

Northeastern region of Brazil were used (Table I). The selected genotypes were cultivated in Seropédica, RJ, Brazil (22º 45' S, 43º 41' W) under the same growing conditions. The mature grains were dried in a forced ventilation oven at 60 °C for 48 h and subsequently lyophilized and ground to a fine powder. The flour obtained was used for all analysis (storage proteins, mineral composition, phytate content and SDS-PAGE).

Subsamples of 100 mg for each of the 3 replicates were taken from a single sample of flour from grains of different plants. The extraction process was sequential so that the precipitate from one extraction was used as the pellet for the following extraction (Figure 1).

Protein determination

Total and storage grain protein fraction (albumin, globulin, prolamin and acid glutelin) contents were estimated by method of Bradford (1976), whereas for the basic glutelin protein fraction the method used resembled that developed by Lowry et al. (1951). Bovine serum albumin (BSA) was used as standard.

Total percentage of nitrogen (N) was determined in triplicate by the Kjeldahl method and the percentage of total protein was calculated by multiplying the grain N content by 6.25.

SDS-PAGE of storage proteins

Electrophoretic analysis was carried out under denaturing conditions (0.1% (w/v) SDS) in 13% PAGE gels. The gels were loaded with 5 µg protein concentration onto each lane, with each lane representing one different genotype. The same process was repeated for all storage protein fractions. The running condition was exactly as described by Schmidt et al. (2015, 2016). The gels were prepared and stained with a solution of silver nitrate as described by Morrissey (1981).

Determination of phytate

Phytate extraction was carried out using a 250 mg sample of flour in 10 mL of 2.4% hydrochloric acid for 3 h at room temperature with constant agitation. The samples were clarified by centrifugation at 6.000 g for 20 min at room temperature. The supernatant was applied and eluted from an anion-exchange resin (Dowex1x8-400, Sigma Co.) and the phytate determination was based on the colorimetric assay described by Latta & Eskin (1980). The assay was performed with 2.0 mL of Wade reagent (0.03% (w/v) $FeCl_3$ and 0.3% sulfosalicylic acid) and 3.0 mL of the eluted sample. The phytate content was determined at 500 nm using a spectrophotometer.

Grain minerals determination

A 100 mg sample of flour was used to determine the minerals content as described by Malavolta et al. (1997). The quantification methods were as follows: metavanadate colorimetric assay for phosphorus (P); atomic absorption spectrophotometry for calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn); flame photometry for potassium (K); and turbidimetry for sulfur (S).

Statistical analysis

Statistical differences among genotypes were compared by means of the Scott-Knott's (Scott & Knott 1974) test ($P< 0.05$) for the quantitative characteristics, using SISVAR software (Ferreira 2008).

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

Genotypes	Origin/Crossing	Growth habitat	Cycle (days)	Grain color	Yield (kg ha^{-1})	Reference
BR17 Gurguéia	BR10 Piauí x CE-315 (Tvu 2331)	semi-branched	75	greenish	976 to 1.695	Freire Filho et al. (1998)
BRS Marataoã	Seridó x TVx1836-013-J	semi-prostate	72 to 77	light greenish brown	933	Freire Filho et al. (2004a)
BRS Milênio	Individual plant selection	semi-prostate	70 to 75	white	1.400	Freire Filho et al. (2009a)
BRS Paraguaçu	BR10 Piauí x Aparecido Moita	branched	65 to 75	white	890 to 1.087	Alcântara et al. (2002)
BRS Acauã	BR10 Gurguéia x Canapu	semi-branched to branched	60	light to yellow	1.338 to 1.407	Santos (2011)
BRS Guariba	IT85F-2687 x TE87-98-8G	semi-erect	65 to 70	white	870	Freire Filho et al. (2004b)
BRS Novaera	TE97-404-1F x TE97-404-3F	semi-erect	65 to 70	white	1.074	Gonçalves (2012)
BRS Pajeú	CNCx 405-17F x TE94-268-3D	semi-prostate	70 to 75	light brown	1.863	Freire Filho et al. (2009b)
BRS Tumucumaque	BRS Guariba x IT87D-611-3	semi-erect	65 to 70	white	1.158,00	Freire Filho et al. (2009c)
BRS Xiquexique	Amapá x BRS Paraguaçu.	semi-prostate	65 to 75	white	1.072,80	Vilarinho et al. (2008)
BRS Sempre Verde	-			light greenish brown	883	Santos & Lima (2015)
IPA 205	-	semi-branched	75	light brown	1.319	IPA (1988)
IPA 206	-	semi-erect	70	light brown	1.240	IPA (1989)
Miranda IPA 207	Vita 3 x CNCx 11-9D	semi-prostate	60 to 68		1.611 to 3.578	Costa et al. (2013)
Epace 10	Seridó x TVu 1888.	semi-branched	65 to 75	brown	1.000	Barreto et al. (1988)
Paulistinha	Local cultivar/ Juazeiro do Norte - CE	-	-	-	1.070	Rocha et al. (2011)
Patativa	EPACE 10	semi-prostate	-	light brown	881 to 2.772	Teixeira et al. (2010)
Pingo de Ouro 1-2	Local cultivar / Iguatu- CE	semi-prostate	-	-	880	Rocha et al. (2011)

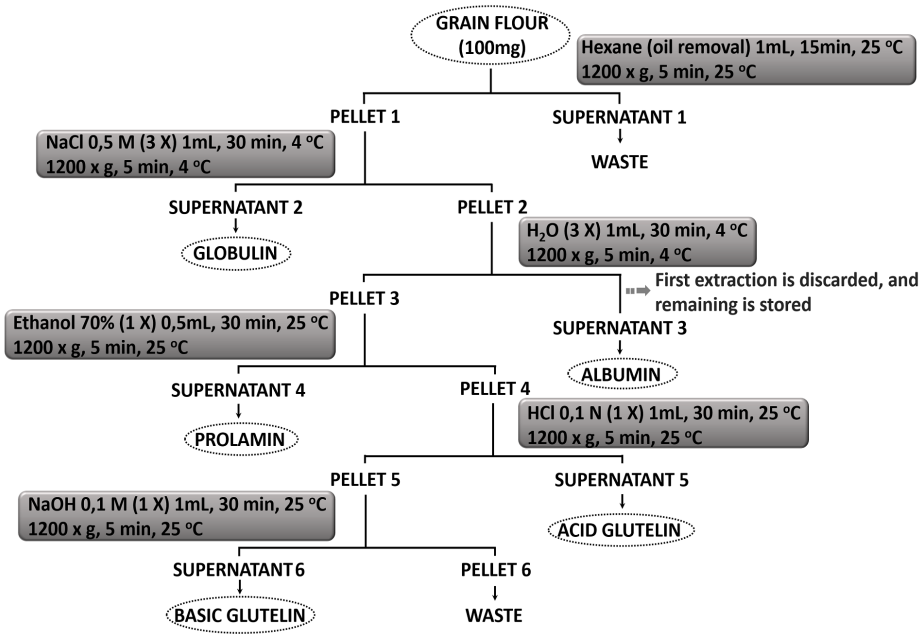

Figure 1. Scheme illustrating the steps of *Vigna unguiculata* (L.) Walp grain storage protein fractioning.

RESULTS

Grain cowpea storage proteins

Significant differences ($P< 0.05$; F's test) among the grain protein fraction averages for albumin and glutelin fractions (basic and acid) (Table II) were observed indicating the existence of genetic variability. However, the Scott-Knott's test did not indicate significant differences for albumins and grouped the basic and acid glutelins in 4 and 2 groups, respectively. All genotypes exhibited higher grain globulin content, followed by basic glutelin, and the lowest content was for prolamin. The albumin fraction exhibited values between 0.257 and 0.684 g $100g^{-1}$ (Table II), thus representing the third group in relative quantity.

SDS-PAGE analysis of the storage protein fractions revealed variations in number and intensity of the protein bands (Figure 2).

The globulin fraction presented a great dispersion in relation to the polypeptides, which varied in molecular mass between 15 and 100 kDa. Genotypes 2, 3, 11, 12, and 14 suppressed the 20 kDa apparent molecular mass polypeptide. The most expressive polypeptides (49 and 50 kDa molecular mass) can be attributed to the vicillins (Fig. 2).

The profile of the albumins showed ten different polypeptides with molecular masses ranging from 10 to 80 kDa. The 25 kDa band exhibited a higher intensity in all genotypes (Figure 2).

The prolamin fraction showed band polymorphism evidenced in genotypes 3, 4 and 12, which did not show the presence of the 30 kDa band, as well as, by another group (genotypes 1, 2, 3, 4, 12, 13 and 14) that presented a pattern of bands with only one polypeptide between 15 and 20 kDa, which is distinct from both that appear within this range in the other genotypes analyzed (Figure 2).

SDS-PAGE for basic and acid glutelins are very similar except for the absence of two polypeptide bands in all genotypes for acid glutelin, once for basic glutelin four polypeptides appeared, two with molecular masses between 15 and 20 kDa, and two with molecular masses between 25 and 30 kDa, whereas for acid glutelin, for the intervals of 15 to 20 kDa and of 20 to 25 kDa, only one polypeptide was detected. In addition, the

Table II. Storage protein soluble fractions contents in grains of the commercial genotypes of *Vigna unguiculata* (L.) Walp. used in this work. The values are expressed as a percentage of the dry flour.

Genotypes	Soluble Fractions					Total Protein
	Globulin	Albumin	Prolamin	Basic Glutelin	Acid Glutelin	
BR17 Gurguéia	21.73[a]	0.51[a]	0.18[a]	2.14[c]	0.90[b]	23.19
BRS Marataoã	24.25[a]	0.57[a]	0.10[a]	1.70[d]	0.63[b]	21.37
BRS Milênio	23.72[a]	0.52[a]	0.11[a]	1.36[d]	0.58[b]	22.53
BRS Paraguaçu	23.40[a]	0.50[a]	0.14[a]	1.64[d]	0.69[b]	24.76
BRS Acauã	21.08[a]	0.42[a]	0.14[a]	3.80[a]	1.29[a]	23.88
IPA 205	21.64[a]	0.26[a]	0.18[a]	4.14[a]	1.61[a]	25.42
IPA 206	21.50[a]	0.32[a]	0.18[a]	3.90[a]	1.41[a]	24.27
Patativa	25.23[a]	0.50[a]	0.20[a]	3.25[b]	1.30[a]	23.98
Pingo de Ouro 1-2	23.11[a]	0.41[a]	0.11[a]	2.60[c]	1.03[a]	21.86
Epace 10	26.01[a]	0.50[a]	0.18[a]	3.56[b]	1.19[a]	25.14
BRS Guariba	27.81[a]	0.68[a]	0.15[a]	1.44[d]	0.57[b]	25.81
BRS Novaera	25.96[a]	0.68[a]	0.15[a]	1.35[d]	0.52[b]	22.49
BRS Pajeú	24.43[a]	0.45[a]	0.15[a]	4.07[a]	1.27[a]	24.46
BRS Tumucumaque	27.71[a]	0.67[a]	0.12[a]	1.62[d]	0.54[b]	25.46
BRS Xiquexique	24.74[a]	0.38[a]	0.16[a]	3.29[b]	1.30[a]	24.74
IPA 207	23.16[a]	0.35[a]	0.18[a]	3.86[a]	1.34[a]	28.56
Paulistinha	28.10[a]	0.53[a]	0.16[a]	3.35[b]	1.22[a]	29.20
BRS Sempre Verde	24.26[a]	0.46[a]	0.17[a]	2.71[c]	1.01[a]	23.60
SE	1.7926	0.0808	0.0201	0.2109	0.1287	-
P>Fc	0.1378	0.0148**	0.1546	0**	0**	-
CV(%)	14.74	33.41	28.29	25.14	15.25	-

***Averages with the same letter, in column, belong to the same Scott-Knott grouping at 5% probability; **Significant in F testing at 5% probability; SE= standard error; CV=coefficient of variation(%).**

genotypes 2, 3, 10, 11, 12 and 14 showed bands with higher intensity for basic glutelin between 40 and 70 kDa, whilst genotypes 1 and 8 did not present well-defined bands within the evaluated spectrum (Figure 2).

Among the 18 genotypes, the total grain protein content ranged from 21.4% for BRS Marataoã to 29.2% for Paulistinha (Table II).

Grain minerals and phytate

Zn content varied from 46.75 to 64.25 mg kg^{-1} for Pingo de Ouro 1-2 and BRS Paraguaçu genotypes, respectively, while for Fethere was no difference among the 18 genotypes studied, ranging from 58.5 to 69 mg kg^{-1} (Table III). BRS Paraguaçu with high Zn content and the Paulistinha with high Mn content, also exhibited high contentsof K, Ca and S. The phytate concentration in the grain ranged from 6.76 to 12.22 mg g^{-1} of flour (Table III).

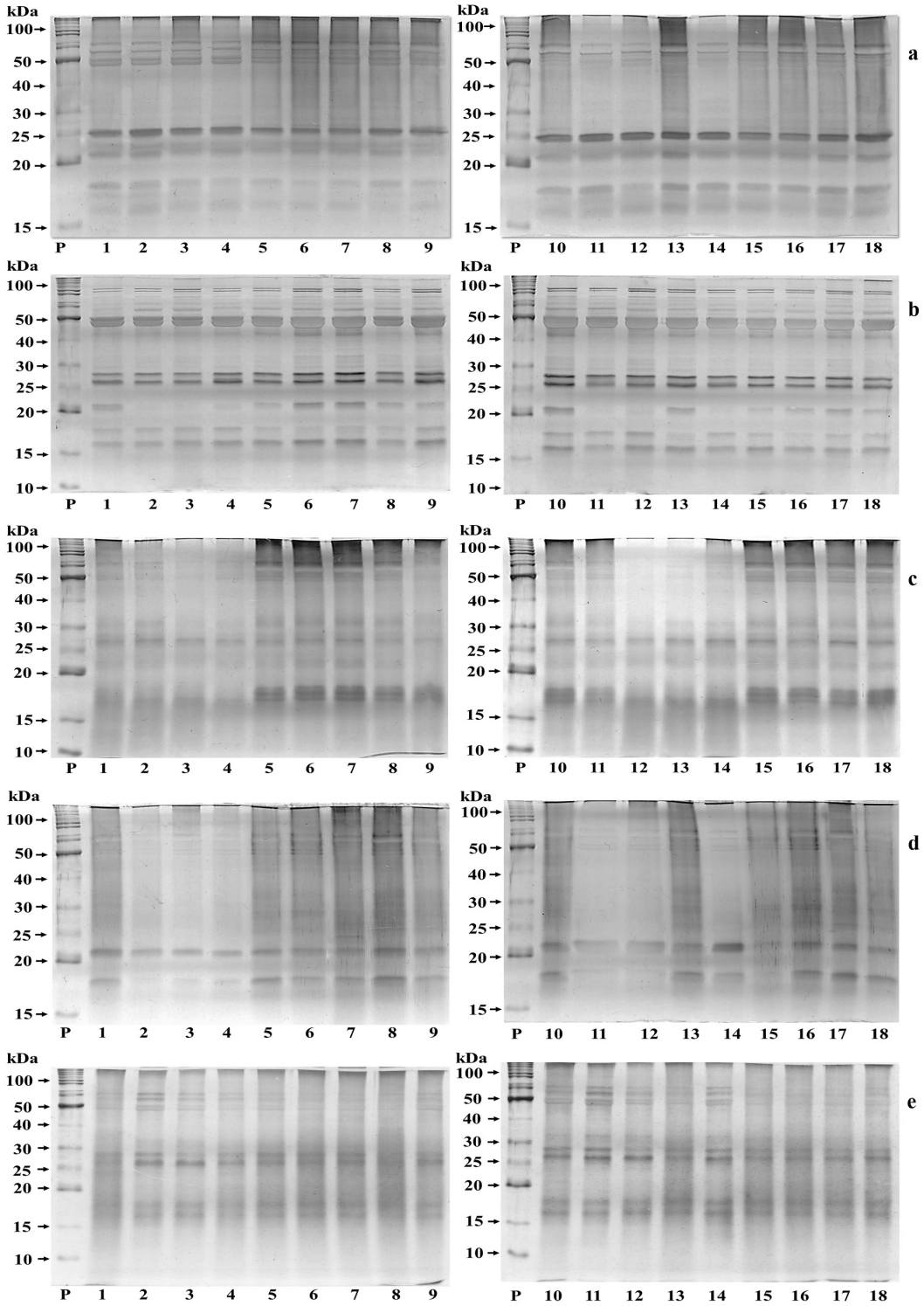

Figure 2. Electrophoretic SDS-1D-PAGE profiles for: a-Albumins; b-Globulins; c-Prolamins; d-Acid Glutelin; e-Basic Glutelin. Lane "P" - molecular mass standard; 1- BR17 Gurguéia, 2- BRS Marataoã, 3 - BRS Milênio, 4 - BRS Paraguaçu, 5 - BRS Acauã, 6 - IPA 205, 7 - IPA 206, 8 - Patativa, 9 - Pingo de Ouro-1-2, 10 - Epace 10, 11- Guariba, 12 - BRS Novaera, 13 - BRS Pajeú, 14 - BRS Tumucumaque, 15 - BRS Xiquexique, 16 - IPA 207, 17 - Paulistinha, 18 - BRS Sempre Verde.

Table III. Mineral and phytate contents in the grains of the genotypes of *Vigna unguiculata* (L.) Walp used in this work.

Genotypes	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn	Phytate
	g kg^{-1}					mg kg^{-1}				Mg g^{-1}
BR17 Gurguéia	5.04^{a*}	13.39^{a}	0.43^{a}	1.10^{a}	1.47^{a}	5.00^{a}	61.50^{a}	12.00^{c}	52.75^{c}	10.86^{b}
BRS Marataoã	4.99^{a}	12.24^{a}	0.50^{a}	1.05^{a}	1.58^{a}	4.75^{a}	64.25^{a}	11.75^{c}	58.75^{b}	9.94^{b}
BRS Milênio	5.23^{a}	10.33^{c}	0.45^{a}	1.00^{a}	1.54^{a}	4.50^{a}	61.25^{a}	11.25^{d}	54.50^{b}	10.11^{b}
BRS Paraguaçu	4.88^{a}	13.00^{a}	0.43^{a}	1.15^{a}	1.50^{a}	7.00^{a}	65.75^{a}	13.00^{c}	64.25^{a}	10.25^{b}
BRS Acauã	4.43^{a}	11.86^{b}	0.40^{b}	0.95^{a}	1.54^{a}	5.50^{a}	58.50^{a}	9.75^{d}	49.00^{d}	10.47^{b}
IPA 205	5.05^{a}	12.62^{a}	0.33^{b}	1.10^{a}	1.35^{a}	4.75^{a}	69.00^{a}	12.50^{c}	57.00^{b}	10.34^{b}
IPA 206	5.63^{a}	11.47^{b}	0.30^{b}	1.00^{a}	1.02^{b}	5.00^{a}	69.00^{a}	9.75^{d}	52.25^{c}	12.22^{a}
Patativa	5.49^{a}	11.86^{b}	0.38^{b}	1.00^{a}	1.21^{b}	4.50^{a}	67.00^{a}	10.00^{d}	55.50^{b}	11.64^{a}
Pingo de Ouro 1-2	4.54^{a}	12.62^{a}	0.35^{b}	1.10^{a}	0.95^{b}	4.25^{a}	63.00^{a}	8.25^{d}	46.75^{d}	12.22^{a}
Epace 10	5.36^{a}	13.39^{a}	0.43^{a}	1.15^{a}	1.51^{a}	5.25^{a}	60.50^{a}	14.25^{b}	55.00^{b}	9.36^{b}
BRS Guariba	4.99^{a}	11.47^{b}	0.38^{b}	1.25^{a}	1.50^{a}	4.75^{a}	59.00^{a}	13.75^{b}	47.25^{d}	10.74^{b}
BRS Novaera	4.76^{a}	10.33^{c}	0.38^{b}	1.00^{a}	1.31^{a}	4.25^{a}	62.50^{a}	9.75^{d}	54.50^{b}	6.80^{d}
BRS Pajeú	4.81^{a}	11.86^{b}	0.48^{a}	1.05^{a}	1.43^{a}	5.00^{a}	63.00^{a}	11.00^{d}	50.75^{c}	8.98^{c}
BRS Tumucumaque	5.17^{a}	13.00^{a}	0.45^{a}	1.25^{a}	1.12^{b}	5.50^{a}	60.50^{a}	14.00^{b}	53.50^{c}	7.41^{d}
BRS Xiquexique	5.15^{a}	11.47^{b}	0.45^{a}	1.05^{a}	1.36^{a}	5.75^{a}	60.50^{a}	12.50^{c}	50.75^{c}	8.65^{c}
IPA 207	4.94^{a}	12.62^{a}	0.48^{a}	1.00^{a}	1.10^{b}	5.75^{a}	60.75^{a}	13.00^{c}	50.75^{c}	7.17^{d}
Paulistinha	5.31^{a}	13.39^{a}	0.43^{a}	1.15^{a}	1.34^{a}	5.25^{a}	63.75^{a}	16.00^{a}	50.25^{c}	6.76^{d}
BRS Sempre Verde	5.00^{a}	11.86^{b}	0.35^{b}	1.10^{a}	1.36^{a}	5.00^{a}	65.00^{a}	10.50^{d}	53.25^{c}	10.65^{b}
SE	0.1500	0.4031	0.0343	0.0540	0.1271	0.7095	4.3878	0.6236	1.3003	0.4682
P>Fc	0.002**	0.0004**	0.025**	0.0289**	0.0461**	0.6168	0.9134	0**	0**	0**
CV(%)	4.21	4.69	11.90	7.07	13.38	19.69	9.84	7.45	3.46	9.68

***Averages with the same letter, in column, belong to the same Scott-Knott grouping at 5% probability; **Significant in F testing at 5% probability; SE= standard error; CV= coefficient of variation (%).**

DISCUSSION

Grain cowpea storage proteins

The relative proportion of proteins in the grain strongly affects the quality of total grain protein (Johnson & Lay 1974). The proportion of grain soluble fractions observed in this work corroborates that reported by Gupta et al. (2010) who analyzed seven genotypes of cowpea. Although the prevalence of the globulin fraction, as well as the lower content of prolamins agree with the data reported in the literature (Chan & Phillips 1994, Vasconcelos et al. 2010, Gonçalves et al. 2016), the contents found for the albumin fraction in the grains of the genotypes studied were lower than those reported in the literature, although it is commonly cited as the second largest group of legume grain storage proteins, ranging from 2.5% to 28% (Chan & Phillips 1994).

Although albumin is rich in Lys, Cysand Met (Clemente et al. 1998), it is not a consensus in the literature, since there are studies that did not find significant difference for the contents of Lys and Met between albumin and globulin proteins (Chan & Phillips 1994). On the other hand, globulins represent the most abundant storage protein fraction of the cowpea grain and thus more directly responsible for the nutritional value of the grains (Bressani 1985). The vicillins, the most represented globulin proteins of vigna

species, showed 62% amino acid sequence identity with soybean β-conglycinin α' subunit, which proved to be effective in lowering blood cholesterol and triglycerides levels in humans and animals (Ferreira et al. 2018).

Electrophoretic analysis of proteins has been extensively used for a number of purposes (Azevedo et al. 2003) and successively used as a tool in biosystematics studies of cultivated and economically important crop species (Kalloo et al. 2001, Schmidt et al. 2015, 2016, Medici et al. 2018). It is a key information to know the intraspecific variation if protein standards are to be used in addition to species characterization (Fotso et al. 1994). In this work, the storage protein fractions band profiles of the eighteen genotypes revealed variations in number and intensity of the bands. According to Jayathilake et al. (2018) cowpea contains a complex and unique protein profile with an array of seed proteins including globulins (about 16 protein bands), albumins (about 20 protein bands), glutelins (21 protein bands) and prolamin (one protein band).

Leguminous globulins are classified into two types according to their sedimentation coefficient: 7/8 S vicillin type and 11/12 S legumin type (Wang et al. 2003). Vicillins are often single chain proteins without disulfide bonding that aggregate forming trimer of subunits with molecular masses ranging from 45 to 60 kDa (Fotso et al. 1994, Sales et al. 2000). Amaral et al. (2017) identified that in mung beanthe 8S globulin protein comprises four bands corresponding to 61, 48, 29 and 26 kDa polypeptides.

The most expressed polypeptides found in the globulin fraction have molecular masses (49 and 50 kDa) attributed to the vicillins. Similar results were reported by Gupta et al. (2014) and Hojilla-Evangelista et al. (2018). Gupta et al. (2014) evaluating eleven cowpea genotypes reported a variation for globulins between 10 and 125 kDa with a larger proportion of those that were within the range of 35 to 50 kDa. Hojilla-Evangelista et al. (2018), studying common bean observed a predominance of protein bands between 41 and 59 kDa and at approximately 30 kDa. On the other hand, Chan & Phillips (1994) and Odeigah & Osanyinpeju (1996) found the major globulin polypeptides with molecular masses of 65, 64, 58, 56, 50 and 14 kDa.

Kalloo et al. (2001) have also analyzed cowpea storage proteins by SDS-PAGE and concluded that the electrophoresis technique was the most appropriate technique to distinguish varieties and to reveal the molecular heterogeneity of the storage proteins. In electrophoretic studies of cowpea proteins, albumins have exhibited a similar pattern of subunit distribution (Chan & Phillips 1994). In this work, the 25 kDa albumin was a major protein band in all genotypes tested, differing from other authors who indicated the 99, 94, 91, 86, 32 and 30 kDa molecular mass bands as the dominant albumin polypeptides (Chan & Phillips 1994, Fotso et al. 1994).

Globulins and albumins have some polypeptide subunits at about the same or similar molecular masses, which suggests that cowpea globulins and albumins probably have some polypeptide chains of similar molecular mass, or it may be an indication of the extent of the interaction or unavoidable cross-contamination between globulins and albumins (Odeigah & Osanyinpeju 1996). In addition, the variation observed in the concentration of protein fractions in relation to the literature, is also likely to be related to the genetic background, grain protein content in the sample and to the protein extraction and determination methods used. In this case, the proportion found of albumins and globulins in relation to total grain soluble storage protein content may be related to the extraction method, since the method used based on Vasconcelos et al.

(2010), included changes such as the reduction of extractor volumes and the elimination of the dialysis step. Thus, the lower values of albumins could be explained by the fact that in the method used in this study, proteins extracted with NaCl (0.5 M) are defined as globulins, whereas proteins extracted with distilled water are defined as albumins. In the method of Vasconcelos et al. (2010) the albumins and globulins are both extracted by a saline solution (NaCl 0.5 M) and then subsequently separated by dialysis. Thus, the elimination of dialysis may explain and possibly be the major factor for the low contents of albumin found and an overestimation of the globulin values. Yet, these are differences resulted due to the methods used and once all genotypes are subjected to the same methods, the comparison will be valid.

The analysis of the prolamin fraction allowed the identification of polypeptide polymorphism. Chan & Phillips (1994) reported four dominant bands of 105, 62, 59 and 54 kDa for the prolamins, where only the 62 kDa band is coincident with the prolamin band profiles observed in this work. Gupta et al. (2014) found four polypeptides with molecular masses ranging between 7.94 to 56.23 kDa in five of the eleven genotypes evaluated, while the other six genotypes exhibited only one polypeptide of 56.23 kDa.

The results obtained in this work for the electrophoretic profile of glutelins (acid and basic) showed great similarity between basic and acid glutelins, with bands ranging between 15 and 70 kDa. Such range of molecular mass bands was close to that described by Gupta et al. (2014), who reported molecular masses between 10 and 79.43 kDa. A broader and spectrum for glutelins with molecular masses 101, 68, 31, and 29 kDa is in general observed, whereas and a molecular mass range of 62 to 44 kDa had been reported Chan & Phillips (1994).

The use of electrophoresis for grain proteins analysis has allowed the detection of qualitative and quantitative differences between genotypes in several plant species (Ghafoor et al. 2003). Odeigah & Osanyinpeju (1996) used the electrophoresis technique to determine the possibility of correlation with the presence/absence of specific polypeptide bands with specific or general characteristics of the culture (such as insect resistance or shell color). However, the use of 1D-PAGE may not be sufficient to distinguish complex mixtures of proteins, since the separation is made on the basis of the molecular mass only. Therefore, the use of more refine proteomic techniques such as 2D-PAGE, which considers, in addition to the molecular mass, the isoelectric point (Issaq & Veenstra 2008), can in future works for cowpea help in the distinction and identification of a greater number of proteins allowing a better characterization and individualization of the genotypes as already observed for other crop species.

The evaluation of the total grain protein content shows the good representativeness of the genotype groups studied, which exhibited a range of values close to that found in the literature in studies from different research groups with total grain protein content varying from 23.7 to 30.1% (Gupta et al. 2010, Vasconcelos et al. 2010, Avanza et al. 2013, Harmankaya et al. 2016). Thus, it is evident the potential of this crop species for breeding studies, as well as its more widespread characteristic, the high grain protein content that makes cowpea an attractive source of protein to replace those of animal origin, particularly in regions where it is normally and more widely cultivated and used.

However, it is important to point out that 5.0–37.0% of the total protein in cowpea (mainly globulins) has been reported to be nutritionally unavailable (Gonçalves et al. 2016). Nonetheless,

bioactive peptides with antioxidant activity are successfully obtained from enzymatic proteolysis of cowpea proteins, indicating an interesting potential to be used as functional food ingredients (Gonçalves et al. 2016). Thus, there is still a gap in studies involving cowpea proteins and their true role in human nutrition. What is known today is that cowpea proteins, like other vegetables, are cheaper, require less energy, land and water resources than the production of animal protein. Thus, the emphasis on plant proteins may also result in positive ecological impact (Peyrano et al. 2016). Yet, it is important to characterize the genotypes available and for their use not only in breeding programs, but also to adapt them to the region where it is consumed.

Grain minerals and phytate

Cowpea grains are an excellent source of Ca, Fe and Zn, which are highly desirable from a nutritional perspective, nevertheless these elements may also result in unwanted characteristics such as increased grain hardness and cooking time (Singh et al. 2007). Studies have shown a large variation in the content of these nutrients in cowpea grains reporting contents ranging from 8.1 to 118 mg kg^{-1} for Zn, 6.9 to 218 mg kg^{-1} for Fe, 0.38 and 10.62 g kg^{-1} for Ca, and for K, the most abundant mineral in cowpea grains, between 1.9 and 28.9 g kg^{-1} (Gonçalves et al. 2016). Such wide ranges in concentrations agree with what was observed in this study, however, for Fe and Zn contents, the values observed in this study differ from those reported by Carvalho et al. (2012), who reported higher values for Fe and lower for Zn.

The BRS Paraguaçu genotype with high grain Zn content and the Paulistinha genotype with high Mn content have both also exhibited high K, Ca and S content. The K and Zn contents observed were higher than those reported by Harmankaya et al. (2016) who evaluated three cowpea genotypes for mineral content and found K, P, Ca, S, Mg, Fe, Zn, Mn and Cu amounts adequate to meet macronutrient and micronutrient demands for the human diet according to what is recommended by the National Research Council (1989).

Two of the 18 genotypes studied (Xiquexique and Tumucumaque) are recommended by EMBRAPA (Brazil) due to their high grain Fe and Zn contents (around 60.57 and 51.63 mg kg^{-1}, respectively), whose concentrations are similar values to those found in this study. The Xiquexique and Tumucumaque genotypes did not differ from the other studied genotypes for Fe content, while for Zn the genotypes BRS Marataoã, BRS Milênio, BRS Paraguaçu, IPA-205, Patativa, Epace-10 and BRS Novaera showed greater contents. Thus, these results demonstrated that all genotypes under study could be recommended for this purpose.

The identification of genotypes exhibiting high or low mineral grain contents is important because they can be used in comparative studies to decipher the underlying genetic and physiological mechanisms regulating mineral transport for grain development, as well as to evaluate if the increase of one mineral influences the concentration of any other (Wang et al. 2003). In a study of mineral correlation with homozygous lines of *Phaseolus vulgaris*, Beebe et al. (2000) found positive associations among most of the minerals.

Although leguminous grains contain a good content of essential minerals, they also accumulate significant amounts of components that reduce their nutritional value by reducing the bioavailability of nutrients (Sparvoli et al. 2015). Phytate is well known as one of these compounds. According to Kumar et al. (2010) the order based on the capacity of the cation minerals to form complexes with phytate *in*

vitro is: Cu^{2+}> Zn^{+}> Cd^{2+} at pH 3-7. When the bioavailability of Fe is concerned, Hu et al. (2006) did not findany correlation between the phytate content and the amount or bioavailability of Fe in bean grains.

The grain phytate concentration in the genotypes studied was lower than that reported by Sreerama et al. (2012) who found a concentration of 14 mg g^{-1}, which was higher than that reported by Hídvégi & Lásztity (2002), who found 4.2 mg g^{-1} on average. Breeding for reduced levels of phytic acid can result in undesirable effects, such as the reduction of P, protein and mineral elements in the grain (Raboy et al. 1984). According to Coelho et al. (2002) common bean genotypes that exhibited lower grain phytate concentrations, without reduced P content, would be useful in studies on the regulatory control of phytate synthesis in plants. In this study, the lowest grain phytate contents were observed in four different genotypes (BRS Novaera, BRS Tumucumaque, IPA 207 and Paulistinha), and at the same time the P content did not show significant differences.

Variation in phytate content in dry beans usually accounts for much of the variation in protein levels in grains (Coelho et al. 2002). When considering the cowpea genotypes used in this study, there is a clear trend of an inverse relationship between grain phytate content and total protein content, so that genotypes with higher protein contents tend to have lower phytate contents. This result differs from those reported by Chitra et al. (1995) who indicated for legume plant species a direct correlation between high grain protein and high phytate concentrations, although these authors pointed out that the correlation between these two characteristics for pigeon pea and chickpea is low, suggesting that it may be possible to select a low grain phytate line without reducing its protein content.

To date, the correlation between grain phytate and protein contents is not entirely surprising given the association between protein and phytate in protein storage bodies, but the regulatory mechanisms involved are not known. The importance of the phytate-protein complex in human nutrition has not yet been well elucidated (Kumar et al. 2010).

CONCLUSION

The data set produced for grains of 18 cowpea genotypes widely used revealed limited polymorphisms for protein bands and a great similarity among the genotypes for grain soluble storage protein fractions content. For the characteristics of total grain protein, mineral and phytate contents, the Paulistinha genotype exhibited the better performance, with high total protein content and a more homogeneous mineral composition. In addition, this genotype has also exhibited low grain phytate content, which although it can promote health improvements, its interaction with minerals and proteins present in food has not yet been well elucidated.

Acknowledgments

This research work was partially supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Brazil, Grants 2012/50098- 8) and by scholarships and fellowships from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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How to cite

GONÇALVES FV, MEDICI LO, FONSECA MPS, PIMENTEL C, GAZIOLA AS & AZEVEDO RA. 2020. Protein, Phytate and Minerals in Grains of Commercial Cowpea Genotypes. An Acad Bras Cienc 92: e20180484. DOI 10.1590/0001-3765202020180484.

Manuscript received on May 16, 2018;
accepted for publication on December 17, 2018

FABÍOLA V. GONÇALVES[1]
https://orcid.org/0000-0002-5833-1465

LEONARDO O. MEDICI[2]
https://orcid.org/0000-0002-4411-1082

MARCOS PAULO S. DA FONSECA[1]
https://orcid.org/0000-0003-0107-6720

CARLOS PIMENTEL[1]
https://orcid.org/0000-0002-6579-7749

SALETE A. GAZIOLA[3]
https://orcid.org/0000-0003-2382-6684

RICARDO A. AZEVEDO[3]
https://orcid.org/0000-0001-7316-125x

[1]Universidade Federal Rural do Rio de Janeiro, Departamento de Fitotecnia – IA, BR 465, Km 47, 23897-000 Seropédica, RJ, Brazil

[2]Universidade Federal Rural do Rio de Janeiro, Departamento de Ciências Fisiológicas – ICBS, BR 465, Km 47, 23897-000 Seropédica, RJ, Brazil

[3]Universidade de São Paulo, Departamento de Genética, Escola Superior de Agricultura "Luiz de Queiroz", Av. Pádua Dias, 11, Agronomia, 13418-900 Piracicaba, SP, Brazil

Correspondence to: **Fabíola V. Gonçalves**
E-mail: fabiolaufrrj@gmail.com

Author contributions

Fabíola Vieira Gonçalves: designed and performed experiments, analysed data and co-wrote the paper. Leonardo Oliveira Medici and Ricardo Antunes Azevedo: conceived of the presented idea, supervised the project and co-wrote the paper. Marcos Paulo Santos da Fonseca and Salete Aparecida Gaziola: performed laboratory analysis and co-wrote the paper. Carlos Pimentel: supervised the project, provided cowpea samples and co-wrote the paper.