Storage protein profile and amino acid content in wild rice *Oryza glumaepatula*

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Abstract – The objective of this work was to determine the total protein profile and the contents of the four major protein fractions (albumin, globulin, prolamin and glutelin) and of the amino acids in the endosperm of the rice wild species *Oryza glumaepatula*. The experiment was performed with 29 accessions of this species, collected from 13 Brazilian locations, and two commercial cultivars. Protein samples were prepared using dried, polished, and ground grains to obtain homogeneous, dry flour used in the preparation of extracts. *Oryza glumaepatula* accessions were identified with the highest levels of total protein, albumin and glutelin protein fractions, and amino acids (with the exception of tryptophan) in comparison to the two analized rice cultivars. The albumin and glutelin profiles in SDS-Page were distinct between rice cultivars and *O. glumaepatula*. This wild species has the potential to increase the nutritional quality of rice storage protein through interspecific crosses.

Index terms: Oryza sativa, genetic resources, nutritional quality, plant breeding, SDS-Page.

Perfil da proteína de reserva e conteúdo de aminoácidos no arroz silvestre *Oryza glumaepatula*

Resumo – O objetivo deste trabalho foi determinar os perfis de proteína total e o conteúdo das quatro principais frações proteicas (albumina, globulina, prolamina e glutelina) e de aminoácidos no endosperma da espécie de arroz silvestre *Oryza glumaepatula*. O experimento foi realizado com 29 acessos dessa espécie, coletados em 13 locais no Brasil, e duas cultivares comerciais. Amostras de proteínas foram preparadas com grãos secos, polidos e moídos, para obtenção de uma farinha seca e homogênea, usada no preparo dos estratos. Acessos de *O. glumaepatula* foram identificados com os maiores níveis de proteína total, frações proteicas de albumina e glutelina, e aminoácidos (com exceção do triptofano), em comparação às duas cultivares de arroz avaliadas. Os perfis de albumina e glutelina em SDS-PAGE foram distintos entre as cultivares de arroz e *O. glumaepatula*. Essa espécie silvestre tem o potencial de aumentar a qualidade nutricional da proteína de reserva do arroz por meio de cruzamentos interespecíficos.

Termos para indexação: *Oryza sativa*, recursos genéticos, qualidade nutricional, melhoramento de plantas, SDS-Page.

Introduction

The genus *Oryza* has two cultivated species (*Oryza* sativa and *O. glaberrima*) and 21 wild species, which are distributed in tropical and subtropical regions. In Brazil, there are four species of wild rice – *O. glumaepatula* (AA), *O. alta* (CCDD), *O. grandiglumis* (CCDD), and *O. latifolia* (CCDD) (Rangel et al., 2007). Among these four species, *O. glumaepatula* has attracted more interest, especially regarding the possibility of its use as a gene reservoir, since it has a diploid genome, which makes feasible interspecific crossing with the cultivated

species *O. sativa*. In addition, it is adapted to Brazilian soil and climate (Brondani et al., 2005). In Brazil, the development of interspecific lines of *O. sativa* x *O. glumaepatula* began in 1995. These strains, derived from two backcrosses using rice cultivars as recurrent parents, have some agronomically interesting characteristics, such as fast vegetative growth and increased yield potential (Rangel et al., 2007).

Analysis of encoded proteins by the genome, also known as proteome, is a powerful molecular tool for studying complex biological processes in organelles, cells, organs, and tissues (Komatsu & Tanaka, 2005). Rice is a model plant for genomic and proteomic studies, due to its small genome compared with other cereals. In recent years, there has been a significant progress in the identification and cataloging of rice proteins. More than thirteen thousand proteins, expressed in different tissues and organelles, have been detected, out of which 5,755 have been classified and had their functions determined (Komatsu, 2006). Information on the rice proteome is available in databases such as the Rice Proteome Database (National Institute of Agrobiological Sciences, 2002). However, there is little information on grain storage proteins in these databases (Komatsu, 2006).

Storage proteins accumulate in large quantities during seed development and are mainly stored in special organelles called protein bodies (Halford & Shewry, 2002). As shown by Kim et al. (2009), the total storage protein content in *O. sativa* endosperm varies between 4.3 and 18.2%. These storage proteins are divided into four fractions according to their differences in solubility: albumins (soluble in water), globulins (soluble in salts), prolamin (soluble in alcohol), and glutelin (soluble in acidic or basic solutions) (Shotwell & Larkins 1989). Glutelin fraction is the predominant protein in rice endosperm, and is classified according to the molecular weights of α -glutelin (37 kDa) and β -glutelin (20 kDa) (Katsude-Tanaka et al., 2004).

Rice protein is considered to be of good quality because it contains eight out of ten essential amino acids. Compared with other cereals, such as maize and wheat, rice has a high level of lysine, which provides high digestibility and nutritional quality (Huebner et al., 1990). Determining the contents of amino acids in storage proteins is an excellent source of information for planning the development of rice genotypes with higher grain nutritional quality. In Brazil, rice accounts for 14% of the energy and 10% of the protein consumed daily by the population (Naves & Bassinello, 2006). The identification of high-protein *O. glumaepatula* genotypes, and the development of interspecific lines from this species, could further increase protein content and nutritional quality of rice.

The objective of this work was to determine the total protein profiles and the contents of four major protein fractions (albumin, globulin, prolamin and glutelin) and of the amino acids in the endosperm of the rice wild species *O. glumaepatula*.

Materials and Methods

Twenty nine accessions of the wild rice species *Oryza glumaepatula*, collected from five Brazilian states: Goiás (GO), Mato Grosso (MT), Mato Grosso do Sul (MS), Amazonas (AM), and Roraima (RR) were evaluated (Table 1). A total of 200 panicles were collected from each population, with an average of 40 seeds per panicle. Forty seeds of each population were germinated on paper rolls. After one week, seedlings were transplanted to pots and cultivated in greenhouse, in order to increase the availability of seeds, which were stored later in the genebank of Embrapa (Brazilian Agricultural Research Corporation).

Seed samples of each population were analyzed. As controls, the upland rice cultivars BRS Bonança and Primavera, chosen due to their high yield and to

Table 1. Collecting locations and germplasm storage sites ofthe 29 Oryza glumaepatula accessions analyzed.

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Accession	State/City	Storage site
BGA 14280	Goiás/Uruana	Santo Antônio Farm
BGA 14232	Goiás/Itapirapuã	Córrego Fundo Farm
BGA 14233	Goiás/Itapirapuã	Córrego Fundo Farm
BGA 14160	Amazonas	Lake Caiambé - Solimões River
BGA 14162	Amazonas	Lake Tefé - Solimões River
BGA 14170	Amazonas	Lake Caldeirão - Solimões River
BGA 14137	Amazonas	Island Trocarí - Solimões River
BGA 14179	Mato Grosso do Sul	Codraza - Ladário - Paraguai River
BGA 14188	Mato Grosso do Sul	Porto Manga - Taquarí River
BGA 14187	Mato Grosso do Sul	Porto Manga - Taquarí River
BGA 14186	Mato Grosso do Sul	Porto Manga - Taquarí River
BGA 14268	Mato Grosso/Poconé	Transpantaneira Highway
BGA 14270	Mato Grosso/Poconé	Transpantaneira Highway
BGA 14269	Mato Grosso/Poconé	Transpantaneira Highway
BGA 14210	Roraima/Boa Vista	BR-174 Highway, Km 626 ⁽¹⁾
BGA 14204	Roraima/Boa Vista	Santa Glória Farm ⁽¹⁾
BGA 14202	Roraima/Boa Vista	Campo Alegre Farm ⁽¹⁾
BGA 14203	Roraima/Boa Vista	Novo Destino Farm ⁽¹⁾
BGA 14197	Roraima/Boa Vista	Road to Boa Vista/Taiano ⁽¹⁾
BGA 14195	Roraima/Boa Vista	BR 174 Highway, Km 575 ⁽¹⁾
BGA 14200	Roraima/Boa Vista	Campo Alegre Farm ⁽¹⁾
BGA 14198	Roraima/Boa Vista	Road to Vila São Francisco(2)
BGA 14199	Roraima/Boa Vista	Road to Vila São Francisco ⁽²⁾
BGA 14206	Roraima/Cacaraí	National Park Viruá - Capinarana area
BGA 14207	Roraima/Cacaraí	BR 174 Highway - Manaus/Boa Vista
BGA 14201	Roraima/Boa Vista	Campo Alegre Farm ⁽¹⁾
BGA 14196	Roraima/Boa Vista	BR-174 Highway, Km 560 ⁽¹⁾
BGA 14205	Roraima/Alto Alegre	Road São Vicente/Alto Alegre(1)
BGA 14208	Roraima/Normandia	Road Normandia/Surumum ⁽¹⁾
Primavera	Cultivated	-
BRS Bonança	Cultivated	-

⁽¹⁾Vereda de Lavrado. ⁽²⁾Collection roadside

the fact that they are widely cultivated in Brazil, were used. The 29 wild accessions were quantitatively and qualitatively analyzed (SDS-Page) for total protein and major protein fractions. Protein samples were prepared using dried, polished grains, which were ground, and defatted with acetone, to obtain a homogeneous, dry flour for the preparation of extracts. Sequential extraction of total proteins and protein fractions was performed as described by Turley & Ching (1986). Protein levels were quantified as described by Bradford (1976). The quantification of total protein and protein fractions was performed in triplicate, using three independent replicates of each accession, in a completely randomized design. The statistical analysis was carried out using the software Genes (Cruz, 2001). After the analysis of variance, the means were compared using the Scott-Knott test, at 5% probability.

For comparison, a calibration curve with BSA (bovine serum albumin) was done at concentrations from 2.5 to 40 μ g L⁻¹. Physicochemical analysis of the amino acids was performed as described by Hagen et al. (1989).

Preparation of total protein samples for the qualitative analysis was made in denaturing polyacrylamide gel, using 20 mg of flour and 350 μ L of sample buffer [10 mmol L⁻¹ Tris HCl (pH 6.8), 1% β -mercaptoethanol (v/v), 2% SDS (w/v), 3% glycerol (v/v) and bromophenol blue]. Then, samples were boiled for 10 min to denature proteins, in order to run SDS-Page analysis.

For albumin extraction, we used 100 mg of the flour added to 500 µL of 10 mmol L-1 Tris-HCl solution (pH7.5) and 1 mmol L⁻¹ EDTA. This solution was shaken for one hour and centrifuged (13,000 rpm for 15 min at 4°C). The supernatant (albumin) was collected and precipitated with 1.5 mL of cold acetone, homogenized by inversion, and stored overnight in a freezer at -20°C. The solution was centrifuged at 15°C for 15 min, and the supernatant (acetone) was discarded. The pellet (albumin) was stored in a freezer for subsequent analysis on SDS-Page. For globulin extraction, 500 µL of 10 mmol L⁻¹ Tris-HCl (pH 7.5), 1 mmol L⁻¹ EDTA, and 0.5 mol L⁻¹ NaCl were added to the initial flour. This solution was shaken and centrifuged, and the supernatant (globulin) was collected, precipitated with acetone, homogenized and stored overnight in a freezer. The solution was centrifuged, and the supernatant (acetone) discarded. The pellet (globulin) was stored in a freezer for subsequent analysis on SDS-Page. For prolamin extraction, 500 μ L of isopropanol 60% (v/v) was added to the rice flour, as described by Turley & Ching (1986), with modifications. The solution was shaken and centrifuged, and the supernatant (prolamin) was precipitated, homogenized and stored in a freezer overnight. Then, the solution was thawed and centrifuged, and the supernatant (acetone) was discarded. The pellet (prolamin) was stored in a freezer for subsequent analysis on SDS-Page. For glutelin extraction, 700 µL of extraction buffer was added to the flour, as described by Kawakatsu et al. (2008). This solution was shaken vigorously for two hours and centrifuged for 15 min at 4°C. The supernatant (glutelin) was collected in a new tube and precipitated with 1.5 mL of acetone, homogenized by inversion and stored in a freezer overnight. The solution was centrifuged and the supernatant (acetone) was discarded. The pellet (glutelin) was stored in a freezer for subsequent analysis on SDS-Page.

The analyses of total protein and fraction profile were performed in a denaturing polyacrylamide gel (SDS-Page), in a discontinuous buffer system, with 4.5% stacking gel and 14% resolving gel. Gels were subjected to electrophoresis at 80 mA for approximately three hours, using the Rubi SE 600 system (GE Healthcare, Waukesha, WI, USA). Then, gels were stained with coomassie blue and destained in 5% methanol (v/v), 7% acetic acid (v/v) and 88% water. Finally, gels were photographed using a digital camera Sony Cybershot DSC-P200, (Sony Brasil, São Paulo, SP, Brazil). From the images, the protein profiles were compared using the DNA Simdex 3 beta release software program (Scott Archer and GenetX, cid. Jamestown, CO, USA), which determines the molecular weight of each band from the positions of the marker bands at the low-molecular weight pattern using a Amersham (LMW calibration kit for SDS electrophoresis, GE Healthcare São Paulo, SP, Brazil).

Results and Discussion

Significant differences between the accessions of the wild rice species *O. glumaepatula* were found for total protein and protein fractions (Table 2). Total protein levels were divided into four groups, with ranges from 14.94% (wild genotype BGA14280) to 9.07% (BGA14179). The control cultivars BRS Bonança

and Primavera, together with the wild accessions BGA14210, BGA14232, BGA14233, and BGA14179, showed the lowest levels of total protein (Table 2).

Kennedy & Burlingame (2003) analyzed the protein contents of 2,869 genotypes of rice (2,674 *O. sativa* and 195 *O. glaberrima*), and found 8.8% as the mean for *O. sativa*, ranging from 4.5 to 15.9%. The mean score for *O. glaberrima* was 13.6%, ranging from 10.2 to 15.9%, which was similar to the values found here for the wild species *O. glumaepatula*. Cao et al. (2009) found a wide variation in the levels of storage protein

Table 2. Contents of storage protein and of protein fractions in 29 *Oryza glumaepatula* accessions and in two *O. sativa* cultivars⁽¹⁾.

Genotypes	Total protein	n Albumin	Globulin	Prolamin	Glutelin		
			(%)				
BGA14280	14.94a	4.46c	11.26b	6.88a	77.38c		
BGA14187	14.89a	5.83b	9.28c	6.94a	77.93c		
BGA14208	14.53a	3.68d	7.36d	4.62b	84.33a		
BGA14197	14.22a	3.65d	8.10d	5.26b	82.97a		
BGA14201	14.19a	4.78c	7.25d	6.05b	81.90b		
BGA14137	14.06a	3.60d	6.25d	5.42b	84.76a		
BGA14207	13.98a	4.77c	6.62d	5.22b	83.37a		
BGA14200	13.35b	6.18b	8.39c	5.08b	80.32b		
BGA14268	13.13b	4.55c	7.88d	5.91b	81.68c		
BGA14204	12.92b	4.74c	8.52c	6.58a	80.14b		
BGA14205	12.90b	4.00d	7.64d	4.93b	83.42a		
BGA14198	12.81b	3.98d	9.62c	6.19b	80.20b		
BGA14202	12.60b	3.90d	8.11d	5.21b	82.76a		
BGA14160	12.47b	5.38b	12.69b	7.63a	74.28c		
BGA14188	12.42b	5.00b	11.55b	7.11a	76.31c		
BGA14203	12.30b	5.20b	9.03c	6.74a	79.01c		
BGA14269	11.79c	3.33d	6.64d	4.62b	85.40a		
BGA14186	11.50c	4.57c	10.42b	4.94b	80.09b		
BGA14270	11.42c	5.74b	9.75c	5.43b	79.07c		
BGA14162	11.05c	3.86d	14.48a	8.68a	72.96d		
BGA14196	11.01c	4.43c	7.89d	6.01b	81.64b		
BGA14206	10.79c	4.31c	7.15d	6.38a	82.15b		
BGA14170	10.65c	4.63c	8.36c	8.37a	78.64c		
BGA14199	10.50c	5.29b	6.74d	7.31a	80.64b		
BGA14195	10.28c	4.07d	9.07c	3.88b	82.96b		
BGA14210	10.14d	11.07a	7.18d	4.87b	76.86c		
BGA14232	9.81d	4.80c	11.15b	8.01a	76.04c		
BGA14233	9.13d	5.76b	10.71b	6.42a	77.08c		
BGA14179	9.07d	5.20b	12.04b	5.83b	76.93c		
Primavera	8.70d	4.42c	9.27c	6.23a	80.07b		
BRS Bonança	8.65d	5.98b	14.42a	6.13b	73.46c		
		Means					
Wild rice accessions	12.17	4.86	9.01	6.09	80.04		
Cultivars	8.68	5.2	11.85	6.18	76.77		
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⁽¹⁾Means followed by equal letters do not differ by the Scott-Knott test, at 5% probability.

content (7.38–15.41%) in Chinese varieties of rice (*O. sativa*). Silveira et al. (2010) found a range of 4.4–20.2% for storage protein contents of 550 accessions, in the rice Core Collection of Embrapa, with an average of 10.31%, lower than the average found here (12.17%) for *O. glumaepatula*. These authors proposed a classification as to the protein contents: high (\geq 12%), medium (11.9–9%), and low (\leq 8.9%). From the 29 genotypes evaluated in the present study, 16 genotypes had high-total protein contents and 13 had medium-total protein contents (Table 2), according to this classification.

Oryza glumaepatula accessions showed higher levels of albumin (BGA14210) and glutelin (BGA14208, BGA14197, BGA14137, BGA14207, BGA14205, BGA14202 and 14269) than the two evaluated cultivars. However, no difference was found between the high levels of globulin found in the accession BGA14162 and the ones determined in the 'BRS Bonança'. Similarly, 'Primavera' did not differ from the 12 accessions of *O. glumaepatula* (Table 2) with the highest prolamin contents. Other studies reported similar results for protein fractions contents in these *O. sativa* cultivars (Agboola et al., 2005; Cao et al., 2009). These results denote that *O.glumaepatula* equalize, at least, the highest levels of protein fractions found in cultivated rice.

The wild accessions evaluated here had α -glutelin levels at 40, 39, 38, 36, 37 and 35-34 kDa, while BRS Bonança and Primavera cultivars, at 39, 37, and 35 kDa (Figure 1). Jahan et al. (2001) analyzed the total proteins of O. sativa by SDS-Page and found α -glutelin proteins with 39, 38, and 37 kDa. BGA14232 had no α-glutelin of 37 or 38 kDa, showing a profile with differential α -polypeptides. Moreover, this genotype had α -glutelin at 39 kDa, which was also present in BRS Bonança and Primavera cultivars. Alpha-glutelin with 37 kDa was present in 17 genotypes of O. glumaepatula, as well as in the two O. sativa cultivars. Seven wild genotypes showed α -glutelin with 36 kDa, 19 showed α -glutelin with 34-35 kDa, and BGA14137, BGA14208 and BGA14203 had no α -glutelin with sizes of 35 or 36 kDa.

All accessions of *O. glumaepatula* showed a differential α -polypeptide of 40 kDa, which was not found in BRS Bonança and Primavera (Figure 1). When SDS-Page was performed with protein fractions, the results were similar to those found in

electrophoresis for total protein. For instance, the wild genotypes had a-glutelin ranging from 34 to 40 kDa (Figure 2 D). Orvza glumaepatula genotypes had α -glutelin with 40 kDa, which was not found for the two cultivars of O. sativa. Considering the diversity of α -glutelin found in *O. glumaepatula*, we propose the following classification for this fraction: glutelin α -1 (40 kDa); α -2 (39 kDa); α -3 (38 kDa); α -4 (37 kDa); α -5 (36 kDa); and α -6 (34 or 35 kDa). β-glutelin had poor resolution and could not be separated by SDS-Page. However, its molecular weight was 19 to 21 kDa in the wild genotypes and cultivars evaluated: these values were similar to but lower than those reported by Jahan et al. (2001), who found β -glutelin proteins at 23, 22.5, and 22 kDa. The gels have also shown proteins with a molecular mass



Figure 1. SDS-Page of seed total protein, obtained in *Oryza glumaepatula* and in the *O. sativa* cultivars BRS Bonança and Primavera. A: lane 1, BGA14233; lane 2, BGA14232; lane 3, BGA14280; lane 4, BGA14160; lane 5, BGA14162; lane 6, BGA14170; lane 7, BGA14179; lane 8, BGA14186; lane 9, BGA14187; MW, Molecular mass marker in kilodaltons; lane 11, BGA14188; lane 12, 'BRS Bonança'; lane 13, 'Primavera'. Black arrow inside the Figure indicates a differential profile with the absence of α -glutelin at 37 and 38 kDa, shown with the white arrows. The band corresponding to waxy protein is indicated in the gel. B: highlighted α -glutelin region from the gel image A for: lane 1, BGA14232; lane 2, BGA14162; lane 3, BGA14170; lane 4, Primavera.

of approximately 60 kDa; these proteins are likely to be waxy proteins, related to the synthesis of starch (Kawakatsu et al., 2008).

Albumin fraction of O. glumaepatula genotypes showed high (97-52 kDa), medium (41-30 kDa), and low (25-15 kDa) molecular masses. The profile of the albumin fraction was similar among wild genotypes, and different from O. sativa. The wild genotypes showed a band with a molecular mass of approximately 18 kDa, while the cultivars BRS Bonança and Primavera did not showed such proteins, or had them at such low concentrations that they could not be detectable (Figure 2 A). Nevertheless, globulin profile between O. glumaepatula and rice cultivars was similar. Furthermore, the wild genotypes and the rice cultivars showed very intense bands at approximately 25 kDa (Figure 2 B). Chandi & Sogi (2007) also found a wide range of molecular weights for O. sativa, with variations from 24 to 96 kDa for albumin, and from 23 to 118 kDa for globulin.

Prolamin showed a similar band pattern among rice cultivars and wild rice, and was characterized by low-molecular-weight proteins (18–15 kDa) (Figure 2 C). Van Der Borght et al. (2006) found prolamin between 16 and 10 kDa, which were similar to the values observed in 12 *O. glumaepatula* genotypes. Chandi & Sogi (2007) found slightly larger prolamin, with molecular masses of 28–24 kDa.

Accessions of O. glumaepatula showed higher levels of all measured amino acids, in comparison with the two cultivars of O. sativa, except for tryptophan. Considering the average, only methionine and tryptophan showed higher values in O. sativa, while isoleucine had the same mean values between the two species. In both species, there was a predominance of glutamic acid and aspartic acid. A relevant fact is that lysine levels were higher in O. glumaepatula. This amino acid is considered to be important for the digestibility of food and for its nutritional quality (Huebner et al., 1990). The accessions of O. glumaepatula that had the highest levels of amino acids were BGA14207 (for seven amino acids), BGA14208 (for eight amino acids), and BGA14280 (for two amino acids) (Table 3). BGA14207 and BGA14208 came from two distinct populations collected in Roraima state, Brazil; while BGA14280 came from Goiás state. These three accessions can be used in rice breeding programs to increase grain nutritional quality.



Figure 2. SDS-Page of seed protein fractions, extracted in the wild rice *Oryza glumaepatula* and in the *O. sativa* cultivars Bonança and Primavera . MW: molecular mass marker in kilodaltons. A: lane 1, *O. sativa* BRS Bonança; lane 2, *O. sativa* Primavera; lane 3, *O. glumaepatula* BGA14208; lane 4, BGA14207; lane 5, BGA14162; lane 6, BGA14187. B: lane 1, Primavera; lane 2, BRS Bonança; lane 3, BGA14162; lane 4, BGA14280; lane 5, BGA14232; lane 6, BGA14160. C: lane 1, Primavera; lane 2, BRS Bonança; lane 3, BGA14170; lane 4, BGA14179; lane 5, BGA14280; lane 6, BGA14233. D: lane 1, BRS Bonança; lane 2, Primavera; lane 3, BGA14232; lane 4, BGA14187; lane 5, BGA14268; lane 6, BGA14197. White arrow indicates the band at 25 kDa. Black arrow indicates the band at 34 kDa.

Amino acid	Maximum	Minimum	Mean	OS	OG
Aspartic acid	1.31 (BGA14207)	0.84 (B)	1.06	0.87	1.11
Glutamic acid	2.76 (BGA14207)	1.76 (B)	2.23	1.81	2.35
Serine	0.68 (BGA14207)	0.47 (B)	0.58	0.49	0.61
Glycine	0.53 (BGA14207)	0.44 (B)	0.48	0.46	0.48
Histidine	0.37 (BGA14207)	0.22 (B)	0.30	0.23	0.32
Arginine	1.14 (BGA14207)	0.85 (B)	0.96	0.88	0.98
Threonine	0.55 (BGA14207)	0.38 (B)	0.46	0.40	0.48
Alanine	0.71 (BGA14280)	0.55 (B)	0.64	0.57	0.67
Proline	0.60 (BGA14208)	0.46 (B)	0.52	0.48	0.53
Tyrosine	0.72 (BGA14280)	0.49 (B)	0.60	0.53	0.62
Valine	0.78 (BGA14208)	0.56 (B)	0.65	0.57	0.68
Methionine	0.26 (BGA14208)	0.23 (B)	0.23	0.24	0.23
Cysteine	0.34 (BGA14208)	0.17 (P)	0.25	0.19	0.27
Isoleucine	0.59 (BGA14208)	0.50 (B)	0.51	0.51	0.51
Leucine	1.17 (BGA14208)	0.83 (B)	1.00	0.85	1.05
Phenylalanine	0.88 (BGA14208)	0.57 (B)	0.67	0.58	0.70
Lysine	0.50 (BGA14208)	0.33 (P)	0.42	0.34	0.45
Tryptophan	0.16 (BRS Bonança)	0.15 (P)	0.11	0.16	0.10
Total	14.05	9.8	11.68	10.10	12.14

Table 3. Levels of total amino acids (g per 100 g) determined in grains of *Oryza glumaepatula* (OG) and *O. sativa* (OS).

⁽¹⁾In brackets is the identification of genotype in which the value was found. B, 'BRS Bonanza'; P, 'Primavera'.

Conclusions

1. Oryza glumaepatula accessions have high levels of total protein, albumin and glutelin fractions, and amino acids (excepting for tryptophan), equalizing, at least, the highest levels found in the commercial cultivars.

2. Albumin and glutelin profiles are distinct between rice and *O. glumaepatula*

3. *Oryza glumaepatula* has potential to increase the nutritional quality of rice storage protein through interspecific crosses.

Acknowledgments

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), for the scholarship granted; and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for the financial support.

References

AGBOOLA, S.; DARREN, N.G.; MILLS, D. Characterization and functional properties of Australian rice protein isolates. **Journal of Cereal Science**, v.41, p.283-290, 2005. DOI: 10.1016/j. jcs.2004.10.007.

BRADFORD, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of dye binding. **Analytical Biochemistry**, v.72, p.248-254, 1976. DOI: 10.1016/0003-2697(76)90527-3.

BRONDANI, R.P.V.; ZUCCHI, M.I.; BRONDANI, C.; RANGEL, P.H.N.; BORBA, T.C. de O.; RANGEL, P.N.; MAGALHÃES, M.R.; VENCOVSKY, R. Genetic structure of wild rice *Oryza glumaepatula* populations in three Brazilian biomes using microsatellite markers. **Genetica**, v.125, p.115-123, 2005. DOI: 10.1007/s10709-005-4916-4.

CAO, X.; WEN, H.; LI, C.; GU, Z. Differences in functional properties and biochemical characteristics of congenetic rice proteins. **Journal of Cereal Science**, v.50, p.184-189, 2009. DOI: 10.1016/j.jcs.2009.04.009.

CHANDI, G.K.; SOGI, D.S. Biochemical characterization of rice protein fractions. **International Journal of Food Science and Technology**, v.42, p.1357-1362, 2007. DOI: 10.1111/j.1365-2621.2006.01340.x.

CRUZ, C.D. **Programa Genes**: aplicativo computacional em genética e estatística. Viçosa: Universidade Federal de Vicosa, 2001.

HAGEN, S.R.; FROST, B.; AUGUSTIN, J. Precolumn phenylisothiocyanate derivatization and liquid chromatography of amino acids in food. Journal of the Association of Official Analytical Chemists, v.72, p.912-916, 1989.

HALFORD, N.G.; SHEWRY, P.R. Cereal seed storage proteins: structures, properties and role in grain utilization. **Journal of Experimental Botany**, v.53, p.947-958, 2002. DOI: 10.1093/ jexbot/53.370.947.

HUEBNER, F.R.; BIETZ, J.A.; JULIANO, B.O. Rice cultivar identification by high-performance liquid chromatography of endosperm proteins. **Cereal Chemistry**, v.67, p.129-135, 1990.

JAHAN, M.S.; KUMMARU, T.; SATOH, H.; HAMID, A. Variation of glutelin seed storage protein in Bangladesh rice cultivars. **Rice Genetics Newsletter**, v.18, p.43-46, 2001.

KATSUDE-TANAKA, T.; DULDULAO, J.B.A.; KIMURA, Y.; IIDA, S.; YAMAGUCHI, T.; NAKANO, J.; UTSUMI, S. The two subfamilies of rice glutelin differ in both primary and higher-order structures. **Biochimica et Biophysica Acta**, v.1699, p.95-102, 2004.

KAWAKATSU, T.; YAMAMOTO, P.M.; HIROSE, S.; YANO, M.; TAKAIWA, F. Characterization of a new rice glutelin gene *glud-1*

expressed in the starchy endosperm. **Journal of Experimental Botany**, v.59, p.4233-4245, 2008. DOI: 10.1093/jxb/ern265.

KENNEDY, G.; BURLINGAME, B. Analysis of food composition data on rice from a plant genetic resources perspective. **Food Chemistry**, v.80, p.589-596, 2003. DOI: 10.1016/S0308-8146(02)00507-1.

KIM, S.T.; WANG, Y.; KANG, S.Y.; KIM, S.G.; RAKWAL, R.; KIM, Y.C.; KANG, K.Y. Developing rice embryo proteomics reveals essential role for embryonic proteins in regulation of seed germination. **Journal of Proteome Research**, v.8, p.3598-3605, 2009. DOI: 10.1021/pr900358s.

KOMATSU, S. Plant proteomics databases: their status in 2005. **Current Bioinformatics**, v.1, p.33-36, 2006. DOI: 10.2174/157489306775330651.

KOMATSU, S.; TANAKA, N. Rice proteome analysis: a step toward functional analysis of the rice genome. **Proteomics**, v.4, p.938-949, 2005. DOI: 10.1002/pmic.200401040.

NATIONAL INSTITUTE OF AGROBIOLOGICAL SCIENCES. **Rice Proteome Database**. 2002. Available at: http://gene64.dna. affrc.go.jp/RPD/>. Accessed on: 28 Jan. 2012.

NAVES, M.M.V.; BASSINELLO, P.Z. Importância na nutrição humana. In: SANTOS, A.B. dos; STONE, L.F.; VIEIRA, N.R. de A. (Ed.). A cultura do arroz no Brasil. 2.ed. ver. ampl. Santo Antônio de Goiás: Embrapa Arroz e Feijão, 2006. p.17-30.

RANGEL, P.N.; BRONDANI, R.P.V.; RANGEL, P.H.N.; BRONDANI, C. Agronomic and molecular characterization of introgression lines from the interspecific cross *Oryza sativa* (BG 90-2) x *Oryza glumaepatula* (RS-16). **Genetics and Molecular Research**, v.7, p.184-195, 2007. DOI: 10.4238/ vol7-1gmr406.

SHOTWELL, M.A.; LARKINS, B.A. The biochemistry and molecular biology of seed storage proteins. **The Biochemistry of Plants**: a Comprehensive Treatise, v.15, p.297-345, 1989.

SILVEIRA, R.D.D.; SANTOS, K.F.E.N.; MARTIM-DIDONET, C.C.G.; DIDONET, A.D.; BRONDANI, C. Proteínas de reserva de acessos de coleção nuclear de arroz. **Pesquisa Agropecuária Brasileira**, v.45, p.1441-1447, 2010. DOI: 10.1590/S0100-204X2010001200015.

TURLEY, R.H.; CHING, T.M. Storage protein accumulation in 'Scio' barley seed as affected by late foliar applications of nitrogen. **Crop Science**, v.26, p.778-782, 1986. DOI: 10.2135/cropsci1986.0 011183X002600040032x.

VAN DER BORGHT, A.; VANDEPUTTE, G.E.; DERYCKE, V.; BRIJS, K.; DAENEN, G.; DELCOUR, J.A. Extractability and chromatographic separation of rice endosperm proteins. **Journal of Cereal Science**, v.44, p.68-74, 2006. DOI: 10.1016/j. jcs.2006.03.005.

Received on May 30, 2012 and accepted on January 15, 2013