CIÊNCIAS DE ALIMENTOS

Variation in genetic and environmental effects of beta-conglycinin (7S) and glycinin (11S) protein fractions in conventional and GM soybean cultivars grown in Southern Brazil

Efeito de genética e meio ambiente nos teores de frações proteicas beta-conglicinina (7S) e glicinina (11S) em cultivares de soja convencionais e transgênicas cultivadas no sul do Brasil

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Abstract

The objective of this study was to evaluate the effects of genetic and environmental variation on levels of beta-conglycinin and glycinin in commercial soybean cultivars, which were sown in showcases at Embrapa Soja, Londrina, simultaneously in Ponta Grossa, south Brazil. The concentration of protein fractions was determined by densitometry after electrophoresis in 20 cultivars, 14 conventional and 6 transgenic . We observed a significant variation as the ratio of beta-conglycinin (7S) and glycinin (11S) between the cultivars analyzed. Most 11S/7S ratios (2.21) was observed in the BRS 184, Ponta Grossa and lowest (1.31) in the BRS 233, Londrina. On average, transgenic varieties of Ponta Grossa were higher than in Londrina for both, taking into account the amount of protein fractions.
Key words: Soybean, glycinin and beta-conglycinin, protein fractions

Resumo

O objetivo deste estudo foi avaliar os efeitos de genética e meio ambiente na concentração de beta-conglicinina e glicinina em variedades de soja comercial e que foram cultivadas em fazendas experimentais da Embrapa-soja de Londrina e de Ponta Grossa, no sul do Brasil. A concentração das frações proteicas foi determinada por densitometria após análise por eletroforese em 20 cultivares, sendo 14 convencionais e 6 variedades transgênicas. Foram observadas diferenças significativas na razão de beta-conglicinina (7S) e glicinina (11S) nestas variedades cultivadas. A maior razão 11/7S foi observada na variedade BRS 184 de Ponta Grossa e a menor (1,31) na variedade BRS 233 de Londrina. Em média as variedades transgênicas de Ponta Grossa apresentaram maiores relações que aquelas de Londrina, tendo em conta as quantidades de frações proteicas.
Palavras-chave: Soja, frações proteicas, glicinina e beta-conglicinina

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Introduction

Brazil is the second largest producer and exporter of soybeans in the world (AGRIANUAL, 2010), especially in grain, oil and protein. The added value of the product depends on the oil content and grain protein. The amount of protein corresponds to 40% and oil 20% (PIPOLO, 2002). About 70% of the protein corresponding to glycinin (11S) and β-conglycinin (7S) (CARRÃO-PANIZZI, 2008). The 7S globunin, is a trimeric glycoprotein consisting of three types of subunits (α, α' and β) and the 11S fraction has subunits A1aB2, A1bB1b, A2B1a, A3B4 and A5A4B3 (NIELSEN et al., 1995). According to Ogawa et al. (1989), Kitamura (1995) and Moraes (2006) cultivars that have reduced levels of protein fractions 7S, compensate this loss with increased synthesis of 11S protein fractions, which keeps unchanged the total protein content. The 7S protein is not suitable for production of tofu as brittle product form, however, the higher the 11S/7S ratios, the better the quality of the gel. The 7S fraction was reported by Bittercourt et al. (2007) as capable of producing allergic reactions in mice. The 11S fraction is more suitable for tofu, due to its higher content of sulfur amino acids dominated by –S-S- bonds (CARRÃO-PANIZZI, KWANYEN, ERHAN, LOPES, 2008). The two globulins show considerable differences in key functional properties, include gel-making ability, thermal stability and emulsifying capacity. (RENKEMA; KNABBEN; VLIET, 201l). In general, the 11S protein has a better gel formation ability than the 7S globulin.

On the other hand, the 7S protein has a greater emulsifying capacity and emulsion stability than 11S globulin (POYSA, WOODROW, YU, 2005).

Studies on characterization and functional properties of the proteins glycinin and β-conglycinin in different soybean cultivars were carried out (MOLINA; PAPADOULOS; LEDWARD, 2001, RIBLETT et al., 2001, KHATIB et al., 2002, CARRÃO-PANIZZI et al., 2008). Both fractions

are also capable of stimulating activity receptor for low density cholesterol (LDL), (ANTHONY, 2000; ERDMAN, 2000). This study evaluated the effects of genetic and environmental variation on levels of β-conglycinin and glycinin in twenty soybean varieties that were planted simultaneously in two different locations. These commercial cultivars were produced by Embrapa Soybean, and are recommended for sowings in Southern region of Brazil. Among the cultivars BRS 213 and BRS 257 do not present the lipoxygenases L1, L2 and L3, while BRS 242 RR, BRS 244 RR, BRS 245 RR, BRS 246RR, BRS 255 and BRS 256 RR RR are genetically modified. Studies reveal variations in protein and subunits in cultivars with high levels of 7S and 11S (TAKAHASHI et al., 2001). The effects of environment on protein fractions were reported by Murphy and Ressureccion in 1984, and interaction between genotype and environment for the ratios of 11S/7S proteins was also recorded by Helms et al. (1998) Therefore the content of these protein fractions were evaluated in 20 cultivars sowed in the region of Londrina (23º 11'S, altitude 630 m) and Ponta Grossa (25º 09'S and 884 meters altitude)

Materials and Methods

To quantify the storage proteins glycinin (11S) and β-conglycinin (7S), these protein fractions were separated by discontinuous electrophoresis in polyacrylamide gel in denaturing conditions (SDS-PAGE), according to the methodology proposed by Laemmli (1970) photo documented and quantified. The assays were carried out in triplicate, and approximately 10 mg of each sample were placed in tubes of 1.5 ml and added 1.0 mL of extraction buffer (sodium phosphate buffer 0.05 M, pH 7, 6,0.4 M NaCl; β-mercaptoethanol 0.28%. The tubes were maintained in the ultrasonic bath for 45 minutes, after that they were centrifuged for 15 min at 13,400 X g in a microcentrifuge, Eppendorf 34, 15c. The supernatant, 60μL were removed and

added to another tube containing 30 mL of sample buffer (0.1875 M TRIS, 6.9% SDS, 30% glycerol, pH 6.8, 20 mL of bromophenol blue dye, 0.05 mL of 20% and β-mercaptoethanol. The tubes were placed in a water bath at 100 ° C for 5 minutes and applied 25 mL of solution in 13.5% polyacrylamide gel. The electrophoretic separation was conducted at 40 volts until the dye band reached the surface of the separating gel (~ 1.0 h); the voltage was increased to 80 volts and remained to the end of the separation (~ 5h), which was determined as at the 30 min after the dye reach the gel bottom. After separation, gels were removed from the card and placed in staining solution (1.5 g Coomassie Brilliant Blue G, 90 ml of acetic acid, 450 mL of methanol and 460 mL of water) for 12 h, and bleach in a solution of 75 mL of acetic acid, 250 mL of methanol and 675 mL of water for 24 h. After bleach gels,, were stored in 10% glycerol solution until photo-documentation, which

was used for the L-PIX (Locus Biotechnology, São Paulo, Brazil). After being photographed the gels were quantified using Image Quant TL software, v8.0 (GE Healthcare, São Paulo, Brazil), resulting in areas corresponding to each subunit of interest. The subunits of storage proteins were identified based on electrophoretic mobility contained in papers published in the literature (FONTES et al., 1984; YAKLICH, 2001). To convert the area of each subunit into the amount of protein (mg) it was added to each gel 3 g protein standard bovine serum albumin (BSA). The concentration of 7S was obtained by adding the amounts of subunits α ', α and β (Figure 1). The content of 11S subunits was the total A3, and the basic A1aA1bA2A4 (Figure 1). Thus we obtained there a relationship 11S/7S. Statistical analyzes were performed by using ANOVA and means were compared by Tukey Test (5% probability). t

Figure 1. Electrophoretic patterns obtained by SDS-PAGE of protein extracts of the samples BRS 245 RR (1, 2 and 3), BRS 246 RR (5, 6 and 7), BRS 255 RR (8, 9 and 10), BRS 256 RR (11, 12 and 13) grown in Ponta Grossa-PR. The default (4) containing 3 mg of protein bovine serum albumin. The identification of the subunits was based on literature reports (YAKLICH, 2001).
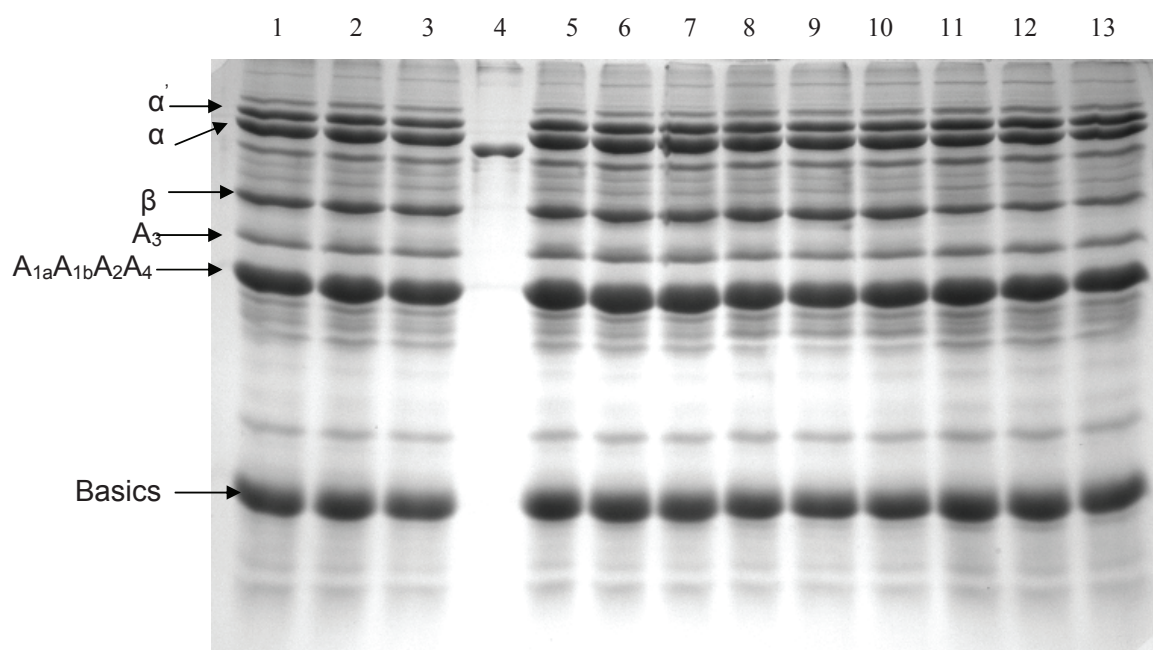
Source: Elaboration of the authors.

Results and Discussion

The relative amounts of the proteins fractions7S and 11S in soybean cultivars sowed in Londrina and Ponta Gross are presented in Tables 1 and 2, respectively. A variation for the 11S/7S ratios were observed between locations and also among cultivars. The 11S protein have high content of sulfur amino acids than 7S subunit so the higher 11S/7S ratio the better nutritional value and the functionality (JI, CAI; CHANG, 1999). An small variability was observed for the mean ratios of 11S/7S among conventional cultivars for both locations 1.65 for Londrina and 1.67 for Ponta Grossa). Transgenic varieties in Londrina presented higher values for 11S/7S ratio (1.95). The same tendency was observed in Ponta Grossa, conventional cultivars shown a average of 1.67 and transgenic cultivars presented an average of 1.74 11S/7S ration . Comparing data from Londrina and Ponta Grossa it was observed larger 11S/7S rations for transgenic varieties in both locations 1.95 to 1.74 respectively. Showing that variations on climate or location had affect these protein fractions. Carrão Panizzi et al. (2008), investigated the genetic variation and environment effects for different Brazilian cultivars and found higher values than those observed in our study, which confirms variability due to different cultivars, soybean season and locations. They also worked with a larger number of soybean genotypes and observed a range of 2.76 for cultivar BRS 169-Bacuri to 1.18 ratio of 11S/7S for cultivar BR-8 Pelotas. Differences among cultivars sowed in Londrina, showed that cultivar BRS 262 presented the lowest content of beta-conglycinin (7S) 5.86, while BRS 233 presented the highest value (10.44) (Table 1). Moraes et al. (2006), analyzing three soybean lines with high protein content (43.17% on average), found values for the relationship of these varieties 11S/7S UFVTN 105, 1.17; 1.85 and 1.87 for isoline 1 and 2. Mujoo, Trinh and Ng (2003) studied seven soybean varieties and found values for the ratio 11S/7S between 1.58 and 2.06 , values compatible with the varieties in this work. The same conventional cultivars when sowed in Ponta Grossa presented 8.49 and 8.97 for 7S protein fraction, considering the range of 7.28 (BRS 184) to 10.99 (BRS 258) (Table 2). For glycinin (11S) protein fraction the highest value observed in Londrina was for cultivar BRS 184 (17.25) and lowest for BRS 259 (11.44) (Table 1). In Ponta Grossa the same cultivars showed 16.03 and 14.14 11S protein fractions, respectively, considering the range for this compound was 114.4 to 16.45 among conventional cultivars (Table 2).

Statistical Procedure

Multivariate analysis can summarize the variability of a complex data set and present it in a more interpretable form. For this reason, Principal Component Analysis of the physicochemical parameters was carried out. The results of this work were confirmed by means of multivariate techniques – PCA which was also applied, to verify the discrimination capacity of the variables of the local groupings (MORRISON. 1990)

Table 1. Subunits of protein fractions by densitometry of polyacrylamide gel (13.5%) in protein of soybean cultivars sowed in Londrina 2009.

Conventional cultivars	α'	α	β	ac3	ac	ba	βconglycinin(7S)	Glycinin (11S)	11S/7S ratio	Total (7S+11S)	Desvia-tions
Embrapa48	2.38abcd	3.26abcd	2.28def	0.90gh	6.85ab	6.99abcde	8.53bcde	14.74bcd	1.73cdef	23.27bcdef	0.41
BRS 184	2.37abcd	3.85a	3.63bc	2.09a	7.45a	7.71a	9.85abc	17.25a	1.75cdef	27.10a	0.19
BRS 213	2.83a	3.78a	3.50cd	1.59cde	7.42a	7.07abcd	10.11ab	16.08ab	1.59efgh	26.19ab	1.16
BRS 232	2.27abcd	3.55ab	4.20ab	2.02ab	6.85ab	6.72abcde	10.02abc	15.60abc	1.56fgh	25.62abc	1.13
BRS 233	2.48ab	3.52ab	4.43a	2.12a	6.15bcde	5.36ghi	10.44a	13.64cdefg	1.31h	24.08abcd	1.08
BRS 257	2.29abcd	3.28abc	4.25ab	1.78abcd	6.44abc	5.48fghi	9.82abc	13.71cdefg	1.39gh	23.53abcde	0.37
BRS 258	2.43abc	2.95cdef	3.08cdef	1.84abc	6.26bcd	5.11hi	8.46bcdef	13.21defgh	1.57fgh	21.67defghi	0.81
BRS 259	2.19abcde	2.84bcde	2.63fg	0.93gh	5.82bcde	4.68i	7.67defgh	11.44h	1.49fgh	19.11ghi	0.53
BRS 260	2.22abcd	3.16abcd	2.98cdefg	1.50cdef	5.84bcdef	6.47bcdef	8.37bcdef	13.82cdef	1.65defg	22.19cdefgh	1.10
BRS 261	2.04bcdef	2.82bcde	1.75hi	0.69h	5.32cde	5.68fgui	6.61fgh	11.69gh	1.76cdef	18.30i	0.61
BRS 262	1.82cdefg	2.51cde	1.53i	0.93gh	5.58cde	5.97efgh	5.86h	12.49efgh	2.13ab	18.35i	0.24
BRS 267	2.47abc	3.06cdef	3.18cdef	1.61bcde	5.85bcde	5.59efgh	8.71abcd	13.06defgh	1.50fgh	21.77defghi	1.43
BRS 268	1.78defg	2.52cde	2.38gh	1.31efg	5.51cde	6.06defgh	6.67efgh	12.88defgh	1.93bcd	19.55ghi	0.33
BRS 282	1.85bcdef	2.57cde	2.78efg	1.38def	5.26de	6.11cdefgh	7.21defgh	12.76defgh	1.77cdef	19.97efghi	0.78
Transgenic cultivars											
BRS242RR	2.05bcdef	2.86bcde	2.34gh	1.50cde	6.03bcde	6.31cdefg	7.26defgh	13.85cdef	1.90bcde	21.11defghi	0.73
BRS244RR	1.54efg	2.32e	2.78efg	1.36ef	5.05e	5.57fghi	6.65efgh	11.99fgh	1.80bcdef	18.64hi	0.60
BRS245RR	1.52fg	2.33e	3.12cdef	1.10fgh	5.17de	7.06abcd	6.98defgh	13.33defgh	1.91bcde	20.31efghi	0.54
BRS246RR	1.18g	2.29e	3.11cdef	1.09fgh	5.10e	7.09abc	6.59fgh	13.29defgh	2.02abc	19.88ghi	0.52
BRS255RR	1.89bcdef	2.94bcde	3.33cde	1.66bcde	5.71bcde	6.81abcde	8.16def	14.18bcde	1.73cdef	22.34cdefg	0.82
BRS256RR	2.57bcdef	2.80de	2.10hi	1.63cde	6.19bcde	7.52ab	7.47gh	15.34bcd	2.33a	22.81defghi	0.93

Means followed by the same letters in the collumns are not different bye Tukey test at 5% de probability.

Source: Multivariate techniques – PCA (MORRISON, 1990).

Table 2. Subunits of protein fractions by densitometry of polyacrylamide gel (13.5%) in protein of soybean cultivars sowed in Ponta Grossa, 2009.

Convencional Cultivars	α'	α	β	ac3	ac	ba	Beta-conglynin(7S)	Glycinin (11S)	11S/7S Ratio	Total 7S+11S	Devia-tions
Embrapa48	2.07ef	2.55e	3.00cdefgh	1.45i	5.67d	7.18abc	7.62ef	14.32ab	1.88bcd	21.94c	0.46
BRS 184	2.01f	2.64de	2.63fghi	2.02cdef	6.10abcd	7.90a	7.28f	16.03ab	2.21a	23.31abc	0.56
BRS 213	2.57abcdef	3.32abcde	3.11cdefg	1.71efghi	5.85cd	7.20abc	9.01bcdef	14.78ab	1.64defgh	23.79abc	0.34
BRS 232	2.14def	2.77cde	3.72abc	2.61a	6.02bcd	7.81ab	8.63cdef	16.45ab	1.90bc	25.08abc	0.41
BRS 233	2.44abcdef	3.62abc	2.91efgh	1.39i	6.93ab	6.94abc	8.97bcdef	15.27ab	1.70cdefgh	24.24abc	0.61
BRS 257	2.39bcdef	3.87ab	2.79efgh	1.56ghi	6.59abcd	6.29c	9.63abcd	14.45ab	1.50ghi	24.08abc	0.82
BRS 258	2.68abcd	3.92ab	4.39a	1.88defgh	6.53abc	6.42c	10.99a	14.84ab	1.35i	25.83abc	0.18
BRS 259	2.48abcdef	4.05ab		1.75defghi	6.09abcd	6.30c	10.26abc	14.14b	1.38i	24.40abc	0.50
BRS 260	2.39bcdef	3.47abcd	2.95defgh	1.48hi	6.26abcd	6.87abc	8.82bcdef	14.62ab	1.65cdefgh	23.44abc	0.67
BRS 261	2.66abcd	3.83ab	2.99cdefgh	1.52hi	6.49abcd	6.56abc	9.50abcd	14.58ab	1.53fghi	24.08abc	0.82
BRS 262	2.56abcde	3.46abcd	2.43ghi	1.76defghi	6.64abcd	7.08abc	8.49def	15.48ab	1.82bcde	23.97abc	0.32
BRS 267	2.86ab	3.64ab	3.35ab	2.31abc	6.20abcd	6.42c	9.86abcd	14.93ab	1.51fghi	24.79abc	1.45
BRS 268	2.26cdef	3.78ab	3.48bcde	1.95cdefg	6.82abc	7.17abc	9.54abcd	15.95ab	1.67cdefgh	25.49abc	1.61
BRS 282	2.47abcdef	3.68ab	2.84efgh	1.67fghi	6.28abcd	6.85abc	9.00bcdef	14.81ab	1.64defgh	23.81abc	0.78
Transgenic cultivars											
BRS 242 RR	2.60abcde	3.68ab	3.04hi	1.99cdef	6.81abc	7.23abc	8.65cdef	16.04ab	1.85bcd	24.69abc	0.76
BRS 244 RR	2.37bcdef	3.66ab	3.04cdefgh	2.15bcd	6.65abcd	7.21abc	9.08bcde	16.02ab	1.76bcdef	25.10abc	0.70
BRS 245 RR	2.41abcdef	3.74ab	3.33bcdef	2.11bcde	7.05ab	7.43abc	9.49abc	16.59a	1.74cdefg	26.08ab	1.15
BRS 246 RR	2.81abc	4.13a	3.46bcde	2.49bcde	7.07a	6.94abc	10.41ab	16.51ab	1.58efghi	26.92a	0.72
BRS 255 RR	2.57abcdef	4.07a	3.67abcd	1.89defgh	6.56abcd	6.93abc	10.31abc	15.38ab	1.49hi	25.69abc	0.59
BRS 256 RR	2.48abcdef	3.08bcde	1.96i	2.04cdef	6.57abcd	6.56bc	7.93ef	15,17ab	2.01ab	22,70bc	0.71

Means followed by the same letters in the collumns are not different bye Tukey test at 5% de probability.
Source: multivariate techniques – PCA (MORRISON, 1990).

Figure 2. Shows the functional relationship and closeness among the variables (protein fraction).
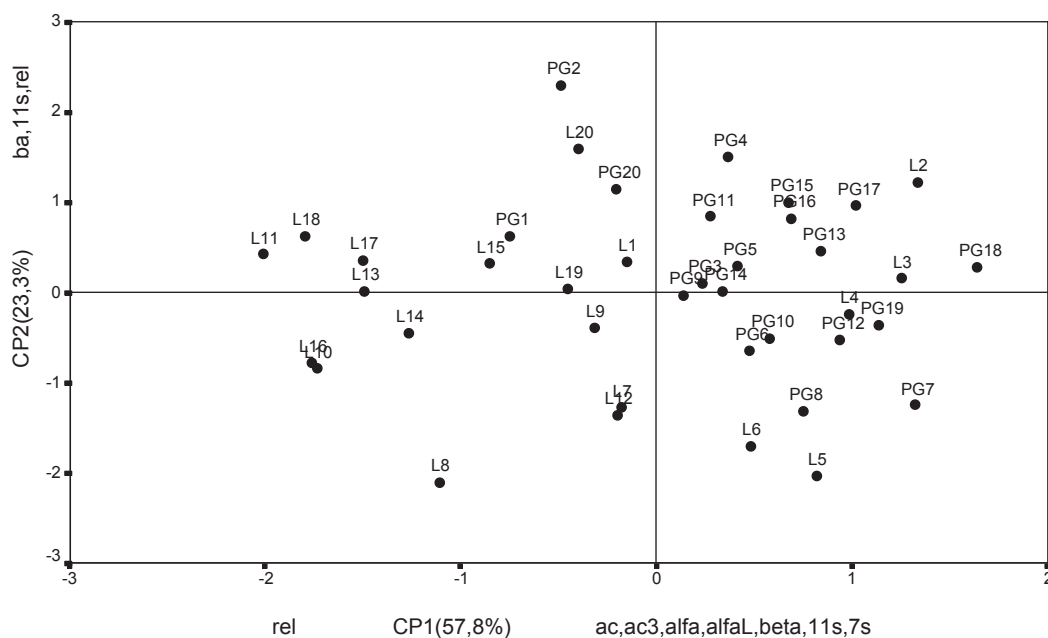
Source: Elaboration of the authors, using program R, Springer, 2011, XII, 306 p.

Figure 1. Samples of soybeab cultivars sowed in Londrina = letter L and in Ponta Grossa =PG.
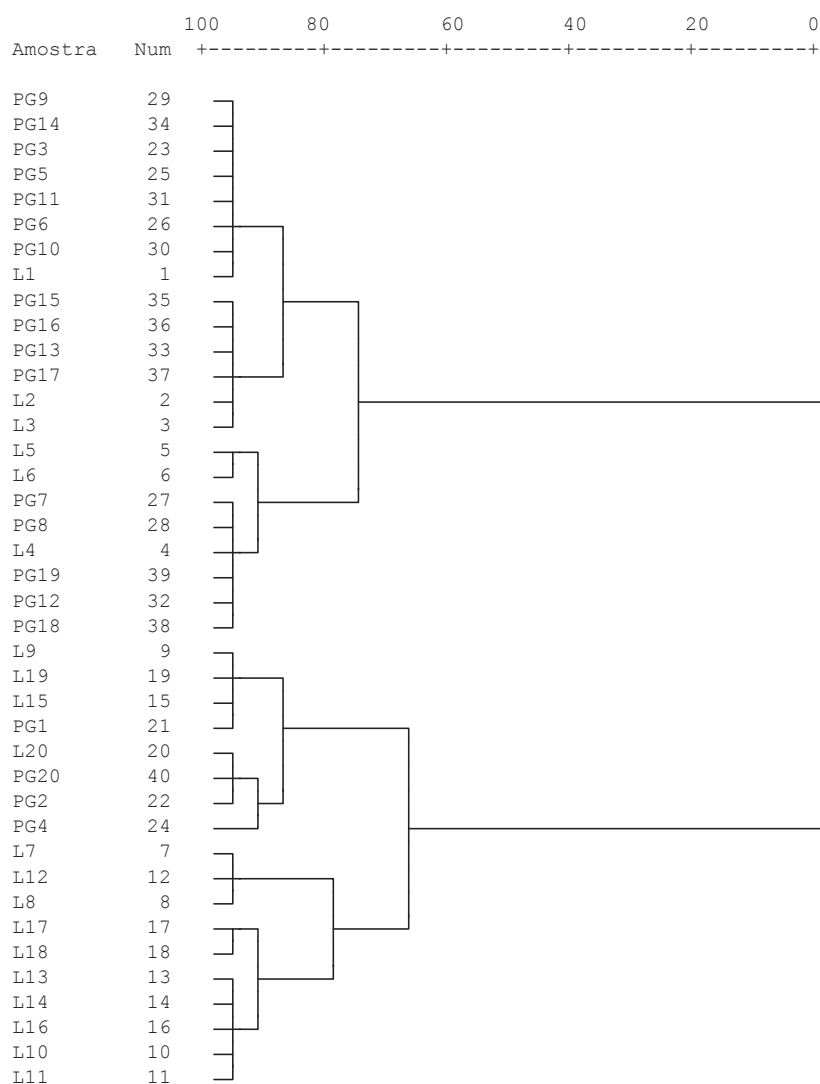
Samples 1 and 21 – Embrapa 48; 2 and 22 – BRS 184; 3 and 23-BRS 213, 4 and 24--BRS 232; 5 and 25- BRS 233; 6 and 26 – BRS 257; 7 and 27-BRS 258, 8 and 28 – BRS 259; 9 and 29 – BRS 260, 10 and 30-BRS261, 11 and 31 – BRS 262, 12 and 32 – BRS 267; 13 and33-BRS 268; 14 and 34 – BRS 282; 15 and 35-BRS 242RR; 16 and 36-BRS 244RR; 17 and 37 – BRS 245RR; 18 and38-BRS 246RR; 19 and 39 – BRS 255RR; 20 and 40 – BRS 2456RR.

This two-dimensional representation provides a scattering of samples, according to their relative position in the plane defined by principal component analysis 1 (PC1) and principal component analysis 2 (PC2) from multivariate statistical analysis. The first component PC1 (horizontal axis), explains 57,8% of the variance and the second component PC2 (longitudinal axis) is associated with 23,3% of the protein fractions. The majority of samples from Ponta Grossa were more influenced by the fractions ac3, alpha, alpha`, beta ad 11S, while samples from Londrina had a greater influence of basic subunit and 11S/7S relation.

Figure 2. shows similarity diagram of the samples by the Ward´s method of highlighting the groups that are represented in Fig. 1.

The cultivars from Londrina, BRS 261, BRS 262, BRS 268, BRS 282, BRS 244RR and BRS 246RR. Showed higher influence of subunit basic and also for total 11S. Variables ac, ac3, alpha´ alpha beta and the most part 11S had lager influence for Ponta Grossa cultivars.

Figure 3. Dendogram for the 20 samples analyzed.

Conclusions

Genetic improvements in search of new varieties allow change in 11S/7S ratios. Theses fractions differ in amino acid profiles and functional properties. 7S proteins present reducing power of serum cholesterol levels. Thus, a breeding to produce changes in 11S/7S ratios may alter functional properties and benefits to human health.

The conventional cultivars of Londrina and Ponta Grossa were not different 11S/7S ratios (1,66 for Londrina and 1,67 for Ponta Grossa). The transgenic cultivars show media 1,90 in Londrina and 1,73 for Ponta Grossa, both with highest than conventional cultivars. The concentration affects the lease for such components, but more intensely in transgenic varieties.

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