UDC 575:633.34
Original scientific paper

THE MAIN SEED STORAGE PROTEINS AMONG HIGH-PROTEIN SOYBEAN GENOTYPES

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Taški - Hajduković K., V. Đorđević, M. Vidić, M. Vujaković, M. Milošević and J. Miladinović (2008): *The main seed storage proteins among high .protein soybean genotypes.*– Genetika, Vol. 40, No. 1, 9-16.

It is known that the main components of the seed storage proteins contribute to the quality of soybean [*Glycine max* (L.) Merr.] food products. The objective of this study was to investigate content of the two of them [glycinin (11S) and β-conglycinin (7S) fractions] and their respective subunits on the new high-protein soybean genotypes from the Institute for Field and Vegetable Crops, Novi Sad, Serbia. Subunits were resolved by SDS-PAGE and gels were analyzed by scanning densitometry.

Out of 20 analyzed genotypes, the β' and β subunits of β-conglycinin were significantly higher in all of the genotypes except KO531 and KO5431. The β subunit of β-conglycinin was significantly higher in genotypes KO535 KO5437, KO534, KO537, KO539, KO5439, KO532, KO5435, KO538, KO5438 and KO533. The acetic polypeptides of glycinin were significantly higher in genotypes KO5439, KO5437, KO5436, KO5438, KO5432, KO5435, KO5433 and KO5434. The basic

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polypeptides of glycinin were not significantly higher only in genotypes KO539, KO536, KO538, KO535 and KO533. In conclusion, it appears that among the new high-protein genotypes there are genotypes with different amount of subunits that should be bread in the future for a desired level of the protein components.

Key words: Glycine max, β-conglycinin, glycinin, high-proteins seeds, soybean, storage proteins

INTRODUCTION

Soybean (Glycine max L. (Merr.) is an important crop because its seeds contain high concentrations of protein and oil. Most of the commercially grown soybean cultivars contain about 40% protein and 20% oil. More recently soybeans have been bred to increase seed yield and oil content because it was mainly used for the oil refine for human consumption, whilst protein meal was used as a source of high quality protein for animal husbandry. Nowadays, with increase in the consumption of meat, the demand for protein in animal husbandry has increased and high percent of the protein fraction in soybean seeds is now attributable. Thus breeders released more and more high seed lines for commercial used.

About 70% of the storage proteins in soybean seed are the salt-soluble proteins termed globulins. The globulins consist of 11S, 7S, and 2S fraction. The 11S globulins are referred to as glycinin and the 7S globulins are termed β-conglycinin (THANH et al., 1978; DERBYSHIRE et al., 1976). Glycinin accounts for about 60% of storage proteins and β-conglycinin the remaining 40%, though there is some variation among soybean cultivars (NIELSEN et al., 1989). Glycinin is hexamer with molecular weight between 320 x 10^3 and 375 x 10^3 (BADLEY et al., 1975). Each hexamer is composed of two trimers consist of tree monomers. The monomers consist of subunits that are composed of specific acidic (A) polypeptide chain linked by disulfide bonding to a specific basic (B) polypeptide chain and can be one of five subunits ($A_{1a}B_{1b}$, A_2B_{1a}, $A_{1b}B_{1a}$, $A_{54}B_3$ and A_3B_4) (YAKLICH, 2001). The β -conglycinin is trimer with molecular weight of 150-175 x 10^3 (THAN et al., 1977). It is formed by various combinations of three nonidentical but homologous polypeptide subunits (α,' α and β) (THAN et al., 1978). The two major storage proteins do not contain many sulfur aminoacids, although glycinin contains more (3 to 4.5%; FUKUSHIMA, 1991) than β-conglycinin (less than 1%; DERBYSHIRE et al., 1976).

In this paper we report our investigation of the subunit composition of main seed storage proteins among soybean genotypes of Institute for Field and Vegetable Crops by using gel electrophoresis and scanning densitometry.

MATERIAL AND METHOD

Plant materials

Twenty soybean genotypes developed at the Institute for Field and Vegetable Crops in Novi Sad, Serbia were used in the study. The trials were carried out at Institute's experimental fields in Rimski Sancevi, where the genotypes were developed. The trials were conducted during the 2005 growing season.

Chemicals and reagents

All chemicals and reagents were either Sigma company (St. Louis, MO) or of analytical grade.

Moisture and protein contents

Moisture and protein content were determined by NIR spectroscopy using PERTEN DA 7000.

Extraction of seed protein

Soybean seeds were ground in a Termomix, Worwerk. Forty mg of seed powder were extracted in 1ml of extraction buffer (0.03 M TRIS-HCl pH 8.0 containing 0.01 M β-mercaptoethanol. The samples were left for 1 hour at room temperature with vortexing every 10 min. The samples were then centrifuged for 20 min at 11000 g at room temperature. The supernatant contained the total soybean proteins.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The protein content of the supernatant was analyzed according to the Bradford method (BRADFORD, 1976) with bovine serum albumin (BSA) as a standard.

Electrophoresis SDS-PAGE was carried out according to the procedures of Laemmli (LAEMMLI, 1970) in 1 mm thick gels with 12.5% (w/v) separating gel and 5% (w/v) stacking gel in a vertical electrophoreses unit (Carl Roth, Germany). One of outside wells was used for molecular weight standards (Wide Molecular Weight Range, SigmaMarkers). Myosin (Mw 205,000), β-Galactosidase (116,000), Phosphorylase b (97,000), Fructose-6-phosphorylase Kinase (84, 000), Albumin (66,000), Glutamic Dehidrogenase (55,000), Ovalbumin (45,000), Glyceraldehyde-3-phosphate Dehydrogenase (36,000), Carbonic Anhydrase (29,000), Trypsinogen (24,000), Trypsin Inhibitor (20,000), α-Lactalbumin (14,200) and Aprotinin (6,500) were used to estimate the molecular weight ranges of polypeptides and to identify the subunits of the major soybean proteins. The last two wells at opposite end of the gel contained proteins from the control soybean line (Vojvodjanka) used for comparison and ratio calculations. In the remaining inside wells samples of specific soybean genotypes were loaded.

Fifty µl of the extract were mixed with 50 µl of SDS-sample buffer (0.15 M TRIS-HCl, pH 6.8, 3% w/v SDS, 5% v/v β-mercaptoethanol, 7% v/v glycerol and 0.03% Bromphenol Blue) and heated for 3 min in a boiling water bath. Solution was cooled to the room temperature and 15 µl of the sample was loaded onto each well. SDS-PAGE was carried out at 25 mA per gel until the tracking day has migrated thought the stacking gel and then at 45 mA per gel until the Bromphenol Blue was at the bottom of the gel. The temperature of 15-20 °C was obtained by circulating tap-water trough the tank buffer.

The gels were stained by incubation in 0.1% Coomassie Brilliant Blue R-250 during 2 hours. When properly stained, the gels were rinsed in gel fixing solution (3 methanol: 1 glacial acetic acid: 6 distilled water).

Quantification of protein fraction by densitometry

The protein bands on the destaned gel were quantitated using ImageJ software. To quantitate the various protein bands from each soybean genotype, the area of specific protein band of a soybean genotype was compared with area of the protein band for control soybean line (Vojvodjanka) and obtained value was relative to that soybean line. The standardized variables were than analyzed by pairwise means comparisons for significance between Afrodita and all other genotypes.

RESULTS AND DISCUSSION

SDS-PAGE profile of soybean seed proteins

In Fig. 1 are shown patterns of total soybean proteins from 10 different high-proteins soybean genotypes on SDS-polyacrylamide gel. The protein bands were similar among all soybean genotypes. The 7S protein fraction was separated into α, α' and β subunits with molecular weight of 81,000, 74,000 and 50,000, respectively. The 11S protein fraction was separated into acidic and basic subunits. The group of polypeptides near the molecular weight of about 35,000 was a major group of acidic polypeptides. The group of protein bands with molecular weigh values of approximately 14,000 represents basic components.

Quantification of 11S and 7S proteins subunits

Densitometric analysis was used to quantify the subunits of two major soybean seed proteins separated by SDS-PAGE (Table 1).

Out of 20 analyzed high proteins genotypes the genotype KO5436 contained the greatest amount of α' subunits and KO5431 contained the least. The α of β-conglycinin was the highest in KO537. All of the genotypes except KO531 and KO5431 contained significantly more α' and α subunits of β-conglycinin than Afrodita. The β subunits of β-conglycinin were the highest in genotype KO535; they were also significantly higher in genotypes KO5437, KO534, KO537, KO539, KO5439, KO532, KO5435, KO538, KO5438 and KO533, respectively.

The genotype KO5439 had the highest value for the acetic polypeptides of glycinin. Those polypeptides were significantly higher in genotypes KO5437, KO5436, KO5438, KO5432, KO5435, KO5433, and KO5434. The basic polypeptides of glycinin were the highest in KO5439, while in the genotypes KO539, KO536, KO538, KO35, and KO533 they did not significantly differ from Afrodita.

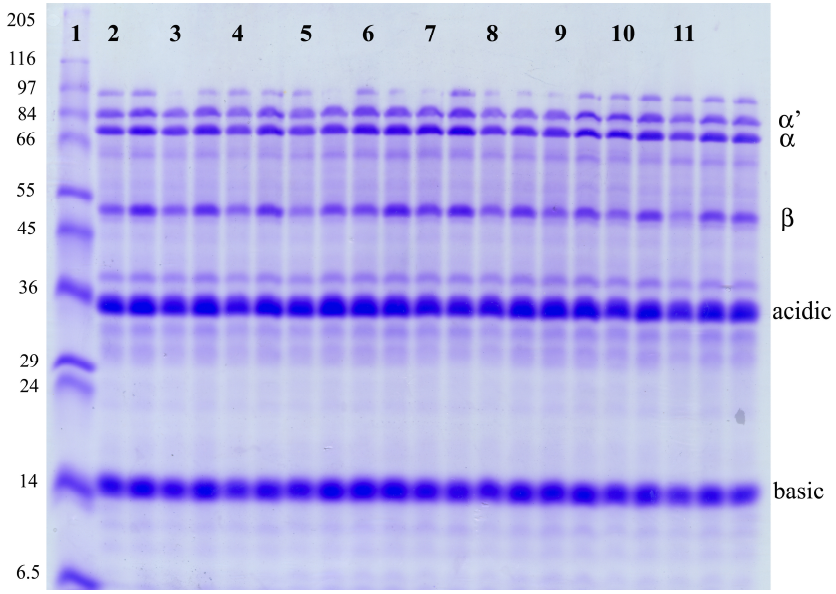

Fig 1 SDS-PAGE gel of the total proteins from soybean genotypes
1. Molecular weight markers (kDa), 2-10 protein profile of some tested soybean genotypes 11. cultivar Vojvodjanka

The most logical reason for increased seed protein apparently resulted from increased quantities of two main storage proteins, glycinin and β-conglycinin (YAKLICH, 2001) and their respective subunits. Comparison of the values for subunits with protein data indicates that KO5436 and KO537 were the highest for the α' and α of β-conglycinin but were only eighteenth and eight highest in protein content, respectively. The β subunits of β-conglycinin were the highest in genotype KO535 which was fifth highest in protein content. In contrast to above mentioned the genotype KO531, which contained the highest total protein content, had subunits of glycinin and β-conglycinin among the lowest of the test genotypes. The data indicated that glycinin and β-conglycinin are increased in the high-protein genotypes but estimating the quantity of subunits of those protein

genotypes did not agree totally with total protein content of the seed. The same conclusion was done by YAKLICH, 2001. WILSON (1987) noted that the quality and quantity of subunits and polypeptides may differ in glycinin and β-conglycinin.

Table 1 Relative protein expression of the subunits of glycinin and β-conglycinin and total seed protein content in high-protein soybean genotypes

	Relative protein expression					
	total	□-conglycinin subunits		glycinin polypeptides		
genotypes	proteins	□'	□	□	acidic	basic
KO531	45.92	1.62	1.32	2.23	1.09	1.75*
KO532	45.42	2.72*	2.03*	2.82*	1.29	2.18*
KO533	44.71	2.62*	2.10*	2.46*	1.09	1.84
KO534	43.17	3.47*	2.15*	2.95*	1.16	2.37*
KO535	43.23	2.56*	2.40*	2.97*	1.01	1.79
KO536	41.77	3.17*	2.39*	2.42	1.06	1.75
KO537	42.53	2.87*	2.66*	2.92*	1.10	1.89*
KO538	41.29	3.13*	2.44*	2.66*	1.24	1.75
KO539	41.54	2.69*	2.14*	2.90*	1.14	1.50
KO5431	41.29	1.55	1.75	2.36	1.13	2.74*
KO5432	40.34	3.23*	2.23*	2.39	1.40*	3.44*
KO5433	43.29	3.44*	2.31*	2.37	1.29*	3.29*
KO5434	40.34	3.62*	2.21*	2.26	1.27*	3.11*
KO5435	41.05	3.4*	2.32*	2.77*	1.33*	2.92*
KO5436	39.70	3.83*	2.30*	2.31	1.44*	3.33*
KO5437	41.69	3.26*	2.18*	2.96*	1.44*	3.21*
KO5438	40.27	2.46*	2.17*	2.46*	1.43*	2.83*
KO5439	42.55	3.11*	2.22*	2.83*	1.87*	2.93*
Afrodita	38.85	1.42	1.14	2.07	1.18	1.49

The asterisks indicate significance between the value and that of Afrodita at P=0.05 according t test

Soybean proteins are known to be deficient in sulfur amino acids, and β-conglycinin is known to contain less sulfur amino acids in comparison to glycinin (WILSON, 1987). Presented data show that in the new high-protein genotypes the amount of glycinin subunits was expressed differently, thus suggesting that some of the tested genotypes could be beneficial in breeding programs aimed at altering composition of seed storage proteins. According to YAKLICH (2001) knowledge of subunit production could significantly affect the quality of protein stored in the soybean seed.

ACKNOWLEDGEMENTS

The authors wish to thank MSc Bojana Stanic for densitometric analysis.

This work was supported by Serbian Ministry of Science, as a part of the project 6852

Received November 26th 2007
Accepted February 28th, 2008

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REZERVNI PROTEINI SEMENA VISOKOPROTEINSKIH GENOTIPOVA SOJE

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I z v o d

Rezervni proteini soje [*Glycine max* (L.) Merr.] imaju veliku nutritivnu vrednost zbog čega imaju veliku primenu u prehrambenoj industriji. Procena sadržaja dva glavna rezervna proteina, glicinina (11S) i β-conglicinina (7S), i njihovih subjudinica kod visokoproteinskih genotipova soje Instituta za ratarstvo i povrtarstvo, Novi Sad uradjena je denzitometrijskim skeniranjem SDS-poliakrilamidnih gelova.

Od 20 analiziranih genotipova, sadržaj α' i α subjedinica β-konglicinina je bio signifikantno veći kod svih, osim kod genotipova KO531 i KO5431. Genotipovi KO535 KO5437, KO534, KO537, KO539, KO5439, KO532, KO5435, KO538, KO5438 i KO533 su imali signifikantno veći sadržaj βsubjedinice β-konglicinina. Sadržaj kiselih subjedinica glicinina je statistički značajno bio viši kod genotipova KO5439, KO5437, KO5436, KO5438, KO5432, KO5435, KO5433 i KO5434. Bazne subjedinice glicinina nisu bile signifikantno povećane samo kod genotipova KO539, KO536, KO538, KO535 i KO533.

Presentovani rezultati pokazuju da visokoproteinski genotipovi imaju značajne razlike u sadržaju polipepditnih subjedinica i da bi neki od njih mogli biti značajni u programu oplemenjivanja na željeni sadržaj proteinskih komponenti rezervnih proteina semena soje.

Primljeno 26. XI. 2007.
Odobreno 28. II. 2008.