Subunit composition of seed storage proteins in high-protein soybean genotypes

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Abstract – The objective of this work was to quantify the accumulation of the major seed storage protein subunits, β-conglycinin and glycinin, and how they influence yield and protein and oil contents in high-protein soybean genotypes. The relative accumulation of subunits was calculated by scanning SDS-PAGE gels using densitometry. The protein content of the tested genotypes was higher than control cultivar in the same maturity group. Several genotypes with improved protein content and with unchanged yield or oil content were developed as a result of new breeding initiatives. This research confirmed that high-protein cultivars accumulate higher amounts of glycinin and β-conglycinin. Genotypes KO5427, KO5428, and KO5429, which accumulated lower quantities of all subunits of glycinin and β-conglycinin, were the only exceptions. Attention should be given to genotypes KO5314 and KO5317, which accumulated significantly higher amounts of both subunits of glycinin, and to genotypes KO5425, KO5319, KO539 and KO536, which accumulated significantly higher amounts of β-conglycinin subunits. These findings suggest that some of the tested genotypes could be beneficial in different breeding programs aimed at the production of agronomically viable plants, yielding high-protein seed with specific composition of storage proteins for specific food applications.

Index terms: *Glycine max,* β-conglycinin, glycinin, protein content, subunit composition.

Composição de subunidades de proteínas de reserva em genótipos de soja com alto teor de proteína

Resumo – O objetivo deste trabalho foi quantificar o acúmulo das principais subunidades de proteínas de reserva da soja, β-conglicinina e glicinina, e como elas influenciam a produtividade e os conteúdos de proteína e de óleo em genótipos de soja com alto conteúdo de proteína. A acumulação relativa de subunidades foi calculada por escaneamento em géis SDS-PAGE, com uso de densitometria. O conteúdo de proteínas dos genótipos testados foi maior que o da cultivar controle dentro do mesmo grupo de maturação. Vários genótipos com conteúdo de proteína aumentado, mas com produtividade ou conteúdo de óleo inalterados, foram desenvolvidos como resultados de novas iniciativas de melhoramento. Esta pesquisa confirmou que as cultivares com alto conteúdo de proteína acumulam maior quantidade de glicinina e β-conglicinina. Os genótipos KO5427, KO5428, e KO5429, que acumularam menor quantidade de todas as subunidades de glicinina e β-conglicinina, foram as únicas exceções. Deve-se atentar para os genótipos KO5314 e KO5317, que acumularam quantidades significativamente maiores das duas subunidades da glicinina, e para os genótipos KO5425, KO539 e KO536, que acumularam quantidades significativamente maiores das subunidades de β-conglicinina. Estes resultados indicam que alguns dos genótipos testados poderiam ser benéficos em programas de melhoramento que visem à produção de plantas agronomicamente viáveis, com sementes com alto conteúdo de proteínas e composição específica de proteínas de reserva para fins alimentícios definidos.

Termos para indexação: *Glycine max,* β-conglicinina, glicinina, conteúdo de proteína, composição das subunidades.

Introduction

Soybean [*Glycine max* L. (Merr.)] is an important source of edible vegetable oil and protein throughout the world and is used in a multitude of food and industrial applications. Seeds of the most commercially grown soybean cultivars contain an average of 360–380 g kg^{-1} protein and 190 g kg^{-1} oil; however, both genetic and environmental factors strongly influence seed composition (Brumm & Hurburgh Junior, 2002; Zarkadas et al., 2007). More recently, soybean genotypes have been bred to increase seed yield and oil content, since they are mainly used for refined oil for human consumption, while

protein meal is mainly used as a source of protein for animal husbandry. With the current increase in meat consumption, the demand for protein in animal husbandry has increased. A major impediment to increasing soybean protein through selective breeding lies in the negative correlation between protein content and yield, on one hand, and oil content and yield on the other (Burton, 1987). Nevertheless, soybean breeders have made notable progress in overcoming that negative correlation and have developed agronomically viable high protein cultivars (Cober & Voldeng, 2000; Wilcox, 2001).

Soybean storage proteins have two major fractions, β-conglycinin (7S) and glycinin (11S), accounting for approximately 70% of the total proteins. Glycinin accounts for about 60% of storage proteins and β-conglycinin for the remaining 40%. β-conglycinin is a glycoprotein with molecular weight of 150–175 x 10^3, and is formed by various combinations of three nonidentical but homologous polypeptide subunits (α', α and β) (Thanh & Shibasaki, 1978). Glycinin is a hexamer with a molecular weight between 320–375 x 10^3. Each subunit consists of an acidic and a basic polypeptide linked by a disulfide bond (Mori et al., 1981).

The major storage proteins do not contain many sulphur aminoacids, although glycinin contains more (3 to 4.5%) than β-conglycinin (less than 1%). Humans and monogastric animals are unable to synthesize sulphur amino acids, and it has been shown that soybean proteins do not provide adequate sulphur amino acids to meet the dietary requirements. As soybean is widely used in animal feeding, and its consumption by humans gradually increases due to the beneficial effect of soybean on human health, improvement of the nutritive value of the globulin ratio in soybean seed became very important. In addition, the functional properties of soybean proteins, as emulsifying or foaming agents for food formulation, are limited by the globular structure of glycinin (Poysa et al., 2006). Such findings provide an opportunity for soybean breeders to develop genotypes with specific protein compositions for specific food applications.

The objective of the present work was to estimate the accumulation of the main seed storage protein subunits, glycinin and β-conglycinin, among high-protein soybean genotypes, and to determine whether these genotypes preferentially accumulate specific polypeptides and how this influences the yield and the protein and oil contents in different maturity groups.

Materials and Methods

Forty soybean genotypes developed at the Institute for Field and Vegetable Crops in Novi Sad, Serbia, were used in the study. The genotypes belonged to maturity groups 0, I, II and III. Each of the groups was represented by nine newly developed high-protein genotypes and one commercially grown cultivar. A commercial cultivar from each appropriate maturity group (with average protein content for high-yielding genotypes) was used as control. Two-year trials were carried out at Institute's experimental fields in Rimski Sancevi. The dominant soil type was calcareous Chernozem with a pH of 7.65 and organic matter content of 3.3%. Field trials were carried out in a completely randomized design, with three replicates and four rows (4 m) per genotype, with 0.45-m spacing between rows. Standard agronomic practices were followed.

Protein and oil content were determined by near-infrared reflectance (NIR) spectroscopy, using PERTEN DA 7000 (Perten, Stockholm, Sweden), with a sample of 250 g of intact soybean seeds. Protein and oil contents were calculated on dry basis.

Soybean seeds were ground in a Thermomix, Worwerk (The Pinehill Partnership Ltd., Berkshire, England). Forty milligrams of seed powder were extracted in 1 mL of extraction buffer (0.03 mol L^{-1} Tris-HCl pH 8.0 containing 0.01 mol L^{-1} β-mercaptoethanol). The samples were left for one hour at room temperature with rotation every 10 min. The samples were then centrifuged for 20 min at 11 x 10^3 *g* at room temperature. The soluble protein concentration in the supernatant was analyzed according to the Bradford method (Bradford, 1976) with bovine serum albumin (BSA) as standard.

Electrophoresis SDS-PAGE was carried out according to the standard procedure (Laemmli, 1970) in 1.5 mm thick gels with 12.5% (w/v) separating gel and 5% (w/v) stacking gel in a vertical electrophoresis unit (Carl Roth GmbH, Karlsruhe, Germany). In one of the outside wells of each gel, molecular weight standards – Wide Molecular Weight Range SigmaMarkers, (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) were run. These markers 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 the opposite end of each gel contained proteins from the soybean cultivar Vojvodjanka, used for comparison and ratio calculations. Samples of the specific soybean genotypes were loaded in the remaining inside wells. Fifty microliters of the extract were mixed with 50µL of SDS-sample buffer (0.15 mol L^{-1} 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. The solution was cooled to 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 dye had migrated through 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 through the tank buffer. The gels were stained with 0.1% Coomassie Brilliant Blue R-250 (Sigma-Aldrich GmbH, Steinheim, Germany) in methanol: acetic acid: distilled water (3:1:6) during two hours. When properly stained, gels were destained in the same solution without dye.

The protein bands on the destained gel were quantified using the ImageJ software (Rasband, 2010). In order to quantify the subunits of seed storage proteins, the specified protein band of the tested genotypes was compared to the same protein band of the Vojvodjanka cultivar. The standardized values were then analyzed by pair wise mean comparisons (t test) for significance between all pairs of genotypes in each maturity group. Principal factor analysis was done using the Statistica 8 (StatSoft, 2010) software. After principal component analysis, factors were rotated by the varimax method.

Results and Discussion

Protein contents differed significantly in all the soybean genotypes studied in all the maturity groups (Table 1). The protein content of the new soybean genotypes, except for KO5319, in all maturity groups was significantly higher than the protein content in the control cultivar of each maturity group. Genotype KO531 had the highest protein content, with about 90 g kg^{-1} more seed protein than the control, followed by genotype KO5427 with ca. 75 g kg^{-1} seed protein. These results agree with those previously reported by Krishnan (2001) and Poysa et al. (2006). Control genotypes of all maturity groups had the highest oil content, but the oil content of high-protein genotypes was similar to that of commercially grown soybean

Table 1. Yield, protein and oil content of high-protein soybean genotypes and control cultivars[(1)].

Genotype	Yield (kg ha^{-1})	Seed protein (g kg^{-1})	Seed oil (g kg^{-1})	Protein/oil ratio
Maturity group 0				
Afrodita	5393±123a	385±0.6e	212±0.3a	1.8±0.01g
KO5439	3614±49d	434±5.0a	174±4.5f	2.5±0.09a
KO5438	4334±93b	408±2.3d	192±2.1cd	2.1±0.03d
KO5437	3969±73c	426±2.9b	189±2.5de	2.3±0.04bc
KO5436	4468±139b	405±4.2d	1972.0±c	2.1±0.04de
KO5435	4560±43b	419±2.9bc	187±1.5de	2.2±0.03c
KO5434	4546±128b	409±2.1d	209±1.2a	1.9±0.02ef
KO5433	4280±42b	435±2.0a	184±0.9e	2.4±0.02b
KO5432	4517±110b	404±1.3d	209±1.4ab	1.9±0.01f
KO5431	4451±134b	412±1.9cd	203±1.0b	2.0±0.01def
Maturity group I				
Balkan	4996±61ab	384±1.5h	211±1.5a	1.8±0.02g
KO5429	4496±55c	436±5.0b	180±3.0e	2.4±0.06b
KO5428	4597±45bc	414±1.3ef	190±0.6d	2.2±0.01de
KO5427	4528±151c	459±1.3a	158±0.4f	2.9±0.01a
KO5426	4797±26abc	404±1.9g	192±1.1cd	2.1±0.01def
KO5425	4403±241c	428±2.5c	183±0.6e	2.3±0.02c
KO5424	4998±174ab	407±2.2g	199±1.9b	2.0±0.03f
KO5423	5177±68a	409±0.7fg	195±0.7c	2.1±0.01ef
KO5422	4798±136abc	419±0.8de	192±0.9cd	2.2±0.01d
KO5421	4471±139c	422±1.6cd	184±1.9e	2.3±0.02c
Maturity group II				
Vojvodjank	5576±159a	379±2.9e	211±1.0a	1.8±0.01g
KO5319	5468±155ab	385±0.1e	208±1.4a	1.8±0.01g
KO5318	4093±180cd	441±4.8a	164±1.2g	2.7±0.04b
KO5317	4651±127bcd	442±2.0a	157±1.0h	2.8±0.02a
KO5316	4822±184abc	433±1.5b	171±0.5f	2.5±0.01c
KO5315	4155±138cd	441±1.7a	176±0.3e	2.5±0.01c
KO5314	4538±212cd	433±1.7b	180±0.9cd	2.4±0.02d
KO5313	4626±190cd	423±1.6c	183±1.5c	2.3±0.01e
KO5312	4004±145d	415±1.9d	192±0.6b	2.2±0.01f
KO5311	4692±195bcd	422±2.1c	178±0.5de	2.4±0.01de
Maturity group III				
Morava	5702±174a	372±2.0f	220±1.6a	1.7±0.02g
KO539	4972±174abcd	416±1.1de	191±0.5b	2.2±0.01ef
KO538	5057±194abc	413±1.3e	191±1.6b	2.1±0.02f
KO537	5360±131ab	421±0.8d	188±0.8cd	2.3±0.01e
KO536	4646±233bcd	418±1.9de	191±1.6bc	2.2±0.02ef
KO535	4572±35cd	436±3.9c	185±1.4d	2.4±0.03d
KO534	4885±194bcd	434±1.0c	173±0.3e	2.5±0.01c
KO533	4244±209d	451±0.9b	160±0.2g	2.8±0.01b
KO532	4696±142bcd	453±1.6b	164±1.1f	2.8±0.02b
KO531	4461±183cd	462±3.4a	157±1.7h	2.9±0.05a

[(1)]Mean±standard errors followed by equal letters do not differ, at 5% probability.

cultivars. Genotypes KO5434, KO5432, and KO5319 remained in the same group as their controls. The oil content varied from 157–220 g kg^{-1}, which was in accordance with the results obtained by Yaklich (2001) and Poysa et al. (2006) for newly developed high-protein soybean genotypes. The highest protein-to-oil ratio was found in genotypes KO531 and KO5427. Except for KO5319, all new soybean genotypes showed significantly higher ratios than the controls.

Yield of the control cultivars was significantly higher than most of the new soybean genotypes, except for genotypes KO5428, KO5426, KO5424, KO5423 and KO5422, in maturity group I, KO5319 and KO5316, in maturity group II, and KO539, KO538, KO537, in maturity group III, which were similar to the controls for the respective maturity groups (Table 1). All genotypes with unchanged yield, except for KO5319, had improved protein content but lower oil content in comparison to the controls. Genotypes KO5434 and KO5432 were the only ones with improved protein content and unchanged oil content, in comparison to the control, but with lower yield. Therefore, these results confirm the well-documented (Burton, 1987; Helms et al., 1998) negative correlations between protein content, yield and oil content.

Patterns of total soybean proteins of some high-protein soybean varieties and cultivar Vojvodjanka on SDS-polyacrylamide gel are shown in Figure 1. The protein bands were similar among all soybean varieties. On the gel, the 7S protein fraction was separated into α', α and β subunits with molecular weights of approximately 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.

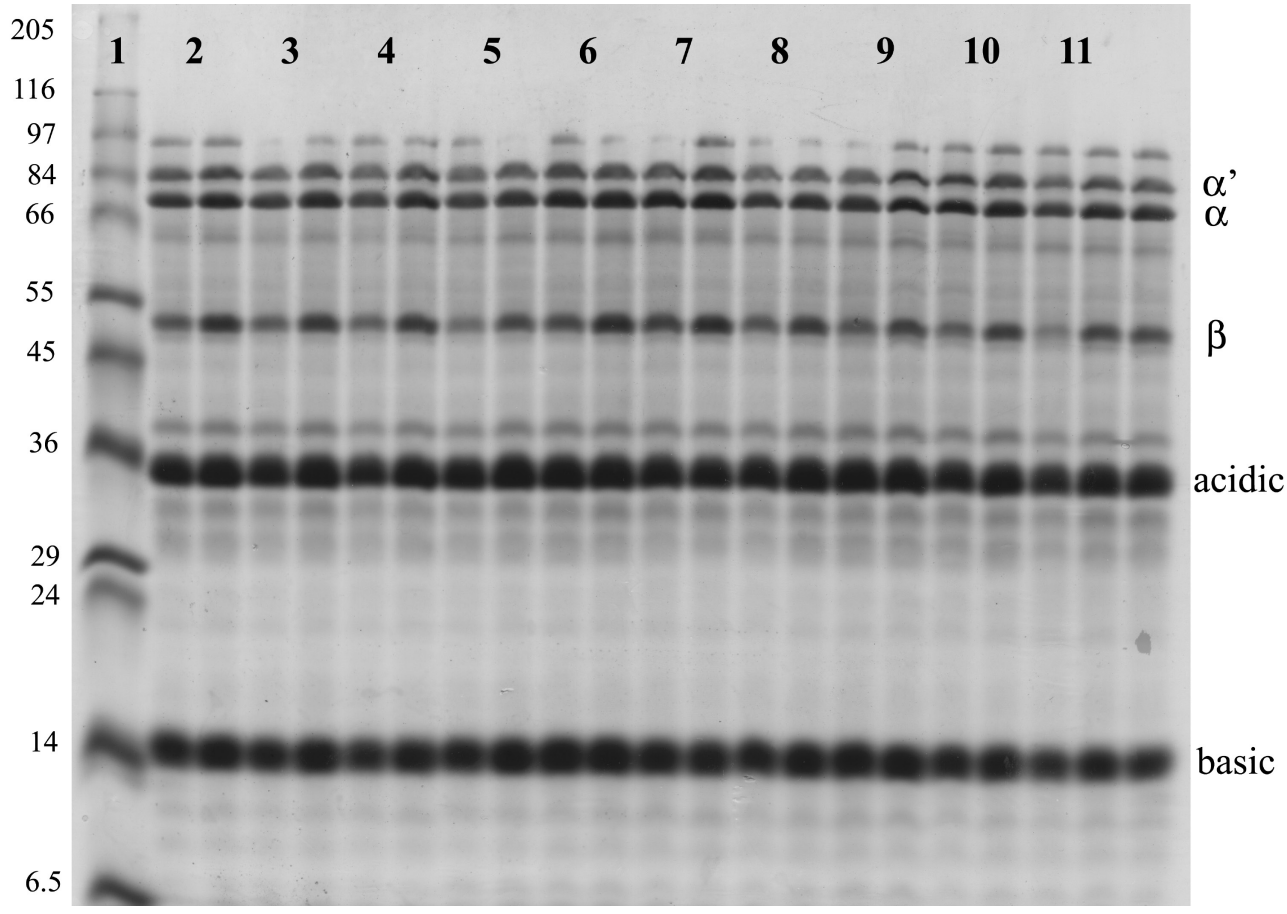

Figure 1. SDS-PAGE gel of the total proteins of high-protein soybean genotypes. 1, molecular weight markers (kDa); 2–10, protein profile of tested soybean genotypes KO5311-KO5319; 11, cultivar Vojvodjanka.

The group of protein bands with molecular weight values of approximately 14,000 represents the basic components. These results corroborate those reported by other authors (Yaklich, 2001; Zarkadas et al., 2007, Taski-Ajdukovic et al., 2008).

Due to their abundance, β-conglycinin and glycinin were the main factors responsible for soybean protein quality. The data in Table 2 show that all genotypes of maturity group 0, except for KO5431, contained significantly more of the α' and α subunits of

Table 2. Relative expressions of seed storage protein subunits in high-protein soybean genotypes and control cultivars[(1)].

Genotype	β-conglycinin			Glycinin	
	α`	α	β	Acidic	Basic
			Maturity group 0		
Afrodita	1.2±0.10d	1.1±0.02b	1.6±0.22b	1.1±0.04e	1.4±0.09f
KO5439	2.6±0.24c	1.9±0.15a	2.2±0.29ab	1.6±0.12a	2.7±0.16de
KO5438	2.5±0.07c	2.2±0.14a	2.1±0.19ab	1.4±0.04bc	2.9±0.09bcd
KO5437	3.2±0.12ab	2.2±0.06a	2.7±0.13a	1.5±0.06ab	3.3±0.04a
KO5436	3.4±0.20a	2.2±0.06a	2.2±0.12ab	1.4±0.05bc	3.2±0.10abc
KO5435	3.4±0.14a	2.1±0.12a	2.5±0.13a	1.3±0.05bc	2.9±0.10bcd
KO5434	3.1±0.25abc	2.0±0.10a	1.8±0.20b	1.1±0.06de	2.8±0.12cde
KO5433	2.7±0.34bc	1.9±0.17a	1.7±0.31b	1.1±0.06de	2.9±0.19cd
KO5432	2.5±0.32c	1.9±0.14a	1.7±0.30b	1.3±0.05cd	3.2±0.09ab
KO5431	1.2±0.15d	1.3±0.19b	1.8±0.26b	1.1±0.05e	2.5±0.18e
			Maturity group I		
Balkan	1.0±0.07e	0.9±0.06d	0.9±0.07d	1.3±0.10e	1.6±0.07c
KO5429	0.7±0.10f	0.7±0.05d	0.7±0.05d	1.0±0.05f	1.0±0.03d
KO5428	0.7±0.01f	0.7±0.01d	0.8±0.05d	0.9±0.03f	1.0±0.02d
KO5427	0.7±0.10f	0.7±0.02d	0.7±0.06d	0.9±0.05f	1.0±0.03d
KO5426	1.5±0.06b	1.6±0.04b	1.9±0.14a	1.7±0.03bc	2.2±0.07a
KO5425	1.2±0.05d	1.1±0.05e	1.5±0.05bc	1.3±0.06e	1.5±0.12c
KO5424	1.5±0.05b	1.5±0.03b	1.5±0.10bc	1.8±0.07b	2.2±0.08a
KO5423	1.4±0.02bc	1.4±0.03bc	1.9±0.12a	1.6±0.04cd	2.0±0.07b
KO5422	1.3±0.6cd	1.3±0.05cd	1.7±0.15abc	1.5±0.04d	1.9±0.08b
KO5421	1.8±0.04a	1.7±0.06a	1.8±0.17ab	2.0±0.04a	2.4±0.09a
			Maturity group II		
Vojvodjanka	1.1±0.03e	1.1±0.03g	1.0±0.02d	1.1±0.04ef	1.1±0.05e
KO5319	1.41±0.01d	1.4±0.02def	1.2±0.06cd	1.3±0.03de	1.4±0.01cde
KO5318	1.4±0.07cd	1.4±0.04cde	1.4±0.07bc	1.5±0.04b	1.8±0.04ab
KO5317	1.1±0.05e	1.2±0.04efg	1.3±0.08bcd	1.3±0.04cd	1.5±0.04bcd
KO5316	1.7±0.07bc	1.7±0.10ab	1.7±0.05ab	1.4±0.10bcd	1.7±0.12abc
KO5315	1.1±0.01e	1.1±0.04fg	1.5±0.30bc	1.1±0.03f	1.3±0.03de
KO5314	1.1±0.03e	1.1±0.02fg	1.0±0.02d	1.3±0.04cd	1.5±0.04bcd
KO5313	1.7±0.16b	1.6±0.16abc	1.7±0.13b	1.5±0.10bc	1.9±0.23a
KO5312	1.6±0.15bcd	1.6±0.17bcd	1.5±0.13bc	1.5±0.07b	1.7±0.06abc
KO5311	2.0±0.05a	1.9±0.04a	2.0±0.15a	1.9±0.06a	1.8±0.21ab
			Maturity group III		
Morava	1.7±0.22cd	1.2±0.07de	0.7±0.05b	1.3±0.07a	1.3±0.03d
KO539	2.9±0.27ab	1.9±0.09abc	2.2±0.31a	1.1±0.10ab	1.3±0.06d
KO538	3.1±0.19a	2.2±0.11ab	2.1±0.27a	1.2±0.10ab	1.7±0.02bc
KO537	2.7±0.17ab	2.4±0.17a	2.4±0.23a	1.1±0.08ab	1.7±0.08abc
KO536	3.1±0.24a	2.0±0.19abc	1.6±0.34a	0.9±0.09b	1.5±0.10cd
KO535	2.5±0.12ab	2.2±0.13ab	2.3±0.32a	1.1±0.11ab	1.7±0.04bc
KO534	2.5±0.45ab	1.6±0.23cd	2.0±0.41a	1.0±0.09ab	1.8±0.23ab
KO533	2.3±0.20bc	1.8±0.15c	2.1±0.20a	1.1±0.13ab	1.8±0.05abc
KO532	2.5±0.12ab	1.8±0.10bc	2.3±0.25a	1.3±0.16ab	2.0±0.08a
KO531	1.5±0.06d	1.2±0.09e	1.7±0.26a	1.0±0.12ab	1.5±0.12cd

[(1)]Mean±standard errors followed by equal letters do not differ, at 5% probability.

β-conglycinin than the Afrodita control cultivar. In maturity group I, there were significantly more α' and α subunits of β-conglycinin than in the control in all genotypes, except for KO5427, KO5428, and KO5429, that had significantly less of those subunits. In maturity group II, there were also significantly more α' and α subunits of β-conglycinin in KO5311, KO5313, KO5316, KO5312, KO5318, and KO5319 genotypes in comparison to the control. Morava, the control cultivar of maturity group III, had significantly lower content of α' subunits compared with all tested genotypes, except for KO531 and KO533. It also had significantly lower content of α subunits except for genotypes KO531 and KO534. Comparing the β subunits of β-conglycinin of high-protein soybean genotypes, of the maturity group 0, to that of the Afrodita normal seed protein line, it was found that only genotypes KO5437 and KO5435 were significantly higher. In maturity group I, these subunits were significantly higher in all genotypes, except for KO5427, KO5428, and KO5429. Genotypes KO5311, KO5316, KO5313, KO5315, KO5312, and KO5318 contained noticeably more β subunits of β-conglycinin than the control cultivar of maturity group II. In maturity group III, these subunits were significantly lower in the Morava control cultivar, when compared to all of the tested genotypes.

Glycinin acetic polypeptides were significantly higher in all tested genotypes, except for KO5431, KO5434, and KO5433 in maturity group 0. In maturity group I, genotypes KO5427, KO5428, and KO5429 contained significantly less acetic polypeptides, while genotypes KO5421, KO5424, KO5426, KO5423, and KO5422 contained significantly more of these subunits than the Balkan control cultivar. Genotypes KO5311, KO5318, KO5312, KO5413, KO5316, KO5317, and KO5314 had significantly higher amounts of acidic polypeptides than the Vojvodjanka control cultivar of maturity group II. Only genotype KO536 from maturity group III differed significantly from the Morava control.

The basic glycinin polypeptides were significantly higher in all genotypes within maturity group 0. Genotypes KO5427, KO5428, and KO5429 had significantly lower amount of acetic subunits, whereas genotypes KO5421, KO5424, KO5426, KO5423, and KO5422 in maturity group I had significantly higher contents. Only genotypes KO5319 and KO5315 from maturity group II and genotypes KO531, KO536, and KO539 did not differ significantly from the controls.

Significant correlations were found among all of the investigated subunits, except for the glycinin acidic subunit, and other protein subunits in maturity group III (Tables 3 and 4). Most of the subunits did not show significant correlations with protein content, except in maturity group I. Only the basic glycinin subunit showed weak significant correlation with the protein content across all the maturity groups. Fehr et al. (2003) observed no correlation between soybean protein subunits and protein content. This finding indicates that it is possible to select soybean genotypes for desired protein composition without influence on protein content. In maturity group 0, all subunits were negatively correlated with yield in contrast to maturity group I, where none of subunits was correlated with yield. Except for maturity group I, β-subunits of β-conglycinin were negatively correlated with yield in all maturity groups.

Earlier studies showed that high-protein cultivars accumulated higher amounts of glycinin and β-conglycinin (Yaklich, 2001; Krishnan et al., 2007). This study confirmed this observation, except for genotypes KO5427,

Table 3. Estimates of phenotypic correlation coefficients among yield, protein and oil contents and protein subunits of soybean genotypes maturity group 0 (above diagonal) and maturity group I (below diagonal), at 5% probability.

	Yield	Protein	Oil	Protein/oil	α` subunit	α subunit	β subunit	Acidic	Basic
Yield		-0.64	0.61	-0.63	-0.42	-0.53	-0.43	-0.62	-0.56
Protein	-0.48		-0.86	0.93	ns	0.26	ns	ns	0.35
Oil	0.49	-0.96		-0.98	-0.26	-0.28	ns	-0.42	ns
Protein/oil	-0.47	0.98	-0.99		ns	ns	ns	0.35	ns
α` subunit	ns	-0.34	0.3	-0.35		0.82	0.67	0.57	0.77
α subunit	ns	-0.38	0.34	-0.39	0.98		0.67	0.67	0.76
β subunit	ns	-0.31	ns	-0.31	0.89	0.92		0.69	0.50
Acidic	ns	-0.38	0.38	-0.41	0.92	0.91	0.76		0.50
Basic	ns	-0.37	0.34	-0.39	0.96	0.96	0.88	0.94	

nsNonsignificant.

KO5428, and KO5429, which accumulated smaller amounts of all subunits of glycinin and β-conglycinin, in comparison to the controls. However, according to the protein content, these genotypes occupied first, sixth, and second place, respectively. This can be the reason why the correlation between protein content and presence of subunits was found to be negative.

Principal factor analyses identified two principal components in maturity groups 0, I and II and three in maturity group III (Figure 2). The first two principal components explain between 72 and 88% of the total variation. All protein subunits were separated by factorial analysis and put aside. Protein content and protein/oil ratio are on the opposite side of yield and oil content on the loading plot, due to their mutual y inverse relationships. No differences between protein subunits in maturity groups 0, I and II were observed. In maturity group III, the acidic subunit was separated from other subunits, and this variation was explained by the third principal component. Expression of acidic subunit of glycinin in high protein genotypes of maturity group III was low, and there was no enhancement of this subunit in comparison to the Morava control cultivar. Principal factor analysis pointed out the interrelationships between yield, protein, oil content, and protein structure and showed the independence of protein subunit content from the other analyzed traits.

Knowledge on subunit production could significantly affect the quality of protein stored in the soybean seed (Yaklich, 2001). Glycinin is a better source of sulphur amino acids than β-conglycinin. The subunits of glycinin in the tested genotypes were expressed differently, indicating that some of them could be used in selection for cultivars with desirable amino acid composition. Attention should be paid to genotypes KO5314 and KO5317, which accumulated significantly higher quantity of both glycinin subunits (basic and acidic) with no significant influence on β-conglycinin. In contrast, genotypes KO5425, KO5319 and KO539 accumulated higher amounts of β-conglycinin subunits with no significant differences on glycinin subunits, and KO536 with significant lower glycinin acetic polypeptides. As reported by Pesic et al. (2005), the level of β-conglycinin had a positive influence on protein extractability. Having this in mind, these genotypes can be used in soybean breeding for the food industry, where greater extractability of protein is needed. It is also important to find a balance between farmers´ demand for high yielding varieties with the demand of the processing industry for enhanced protein content and specific protein composition.

Thus, some genotypes can be selected for future research. Genotype KO5316 has a yield similar to control cultivar Vojvodjanka with higher protein content (approximately 50 g kg^{-1}). Although the yields of genotypes KO5426, KO5424, KO5423 and KO5422 were not significantly different from Balkan, their protein content was about 20 g kg^{-1} higher. An increased protein content in these genotypes was due to enhanced glycinin and β-conglycinin subunits. In maturity group III, genotypes KO537, KO538 and KO539 were not significantly different in yield from the Morava control, but their protein content was 40–50 g kg^{-1} higher. Increase in protein content in the KO537 and KO538 genotypes were due to enhanced β-conglycinin and basic subunits of glycinin. Genotype KO539 accumulates significantly more β-conglycinin than Morava, with no influence on glycinin content.

Table 4. Estimates of phenotypic correlation coefficients among yield, protein and oil contents and protein subunits of soybean genotypes maturity group II (above diagonal) and maturity group III (below diagonal) at 5% probability.

	Yield	Protein	Oil	Protein/oil	α` subunit	α subunit	β subunit	Acidic	Basic
Yield		-0.36	0.4	-0.36	ns	ns	-0.36	ns	ns
Protein	-0.48		-0.92	0.95	ns	ns	ns	ns	0.26
Oil	0.51	-0.97		-0.99	ns	ns	-0.28	-0.27	-0.28
Protein/oil	-0.47	0.98	-0.99		ns	ns	ns	ns	ns
α` subunit	ns	ns	ns	ns		0.86	0.63	0.75	0.64
α subunit	-0.32	ns	ns	ns	0.77		0.73	0.80	0.74
β subunit	-0.64	0.31	-0.33	0.26	0.43	0.72		0.62	0.60
Acidic	ns	ns	ns	ns	ns	ns	ns		0.79
Basic	-0.46	0.34	-0.39	0.35	0.41	0.50	0.71	ns	

nsNonsignificant.

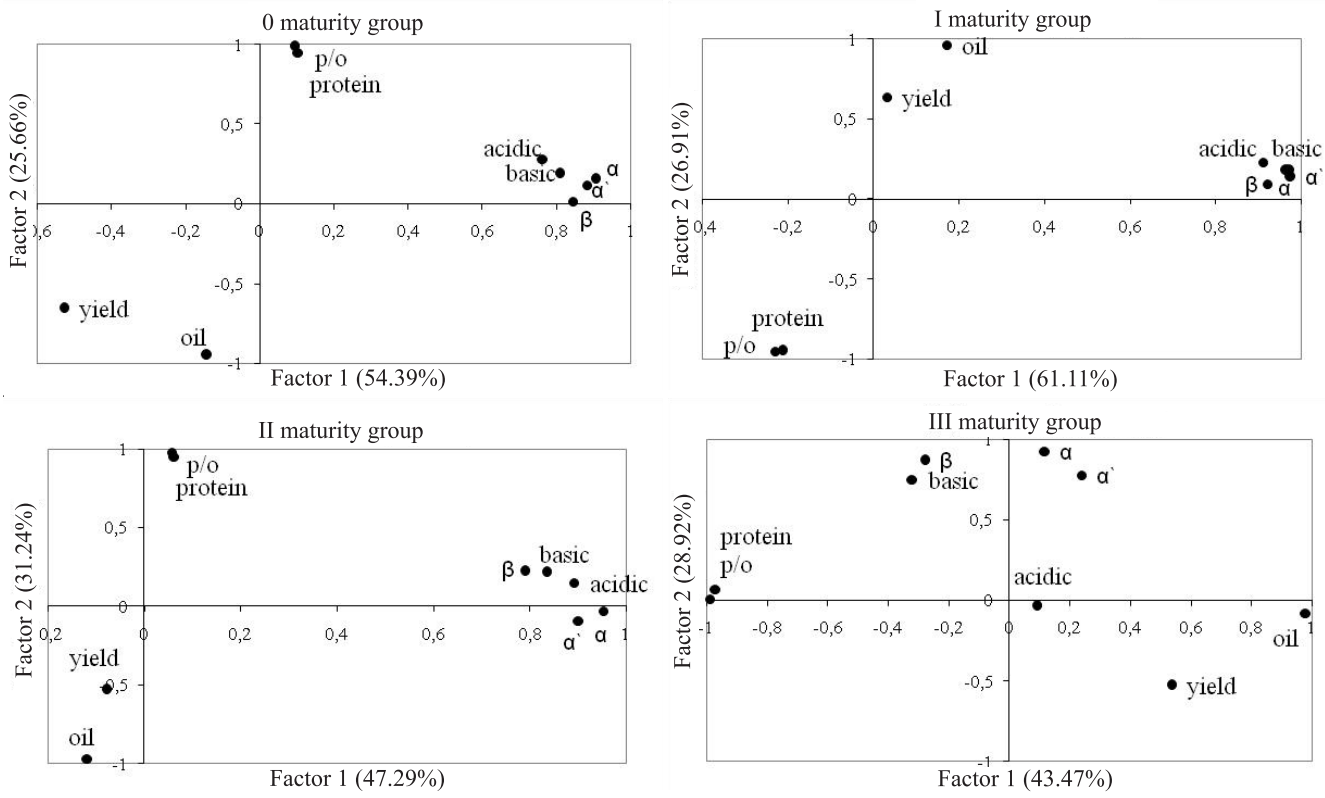

Figure 2. Loading plots of the two first dimensions of principal factor analysis of yield, protein and oil content and ratio and protein subunits.

Conclusions

1. High-protein cultivars generally accumulate higher amounts of glycinin and β-conglycinin.

2. Accumulation of a significantly higher quantity of glycinin subunits, with no significant influence on β-conglycinin, in genotypes KO5314 and KO5317, may be beneficial in selection for cultivars with desirable amino acid composition.

3. Accumulation of higher amounts of subunits of β-conglycinin, in KO5425, KO5319, KO539 and KO536, can be used in soybean breeding for the food industry, where greater extractability of protein is needed.

4. Genotypes KO5426, KO5424, KO5423, KO5422, KO5316, KO537, KO538 and KO539, which have a similar yield to the control for their respective maturity group, can be used in the production of high yielding varieties with enhanced protein content.

5. Protein subunit content is independent of yield, oil and protein content, making it possible to carry out soybean breeding for specific protein composition with desirable agronomic characteristics.

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