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Assessment of seed storage protein composition of six Iranian adopted soybean cultivars [*Glycine max* (L.) Merrill.]

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Abstract
Seed protein quality is an important topic in the production of soybean. The quality of soybean proteins is limited by anti-nutrient proteins and low levels of essential sulfur amino acids. In this study, protein content and solubility of six cultivars were evaluated and seed storage proteins were analyzed using SDS-PAGE and scanning densitometry. The results showed that seed storage protein bands were similar among soybean cultivars. However, concentration of β-conglycinin (7S), glycinin (11S) proteins and related subunits were statistically different among the soybean cultivars. According to the results of this study, 033 and DPX cultivars were characterized by high levels of protein content (42.45 %) and protein solubility (76.58 mg g^{-1}) respectively. Two cultivars DPX and JK were also identified by high 11S/7S ratio (1.39 and 1.43 % respectively). Besides, the JK was considered by the lowest concentration of 7S protein (20.35 %). The results showed that a significant negative correlation existed between protein content and solubility (r= -0.66). A significant and moderate positive correlation was found between acidic and basic subunits with 11S protein (r= 0.72 and 0.47 respectively). The 11S and 7S proteins also showed positive and negative correlation with 11S/7S ratio (r= 0.70 and -0.85 respectively). On the other hand, acidic subunits were characterized by significant positive and negative relationship with 11S/7S ratio and some anti-nutrients protein respectively. Thereupon, these results suggested that the development of new genotypes of soybean with high level of acidic subunits of 11S protein can be notable in increasing seed storage protein quality in soybean breeding programs.

Keywords: Soybean, Seed storage proteins, β-conglycinin, Glycinin, Anti-nutrient proteins.
Abbreviation: 7S, β-conglycinin protein; 11S, Glycinin protein; KTI, Kunitz protein; BBI, Bowman-Birk protein; BSA, bovine serum albumin.

Introduction

The nutritional values of soybean play a prominent role for human and livestock nutrition. Soy protein is increasingly consumed by humans and it also makes a relatively inexpensive protein source for

livestock. However, protein composition of soybean seed is not ideal because of its low levels of the sulfur containing essential amino acids, methionine and cysteine (Fukushima, 1991). Soybean storage proteins mainly consist of globulins, which are classified to 2S, 7S, 11S and 15S according to their sedimentation properties (Osborne and Campbell, 1898). β-Conglycinin (7S) and glycinin (11S) are two major proteins consisting of about 70% of the total seed protein content (Kitamura, 1995). Functional properties of soybean based storage protein are mainly reflected on their composition and structure (Barac *et al*, 2004). Due to abundance of 7S and 11S, these proteins were the main responsible factors for soybean protein quality. The 7S is a glycoprotein and composed of ά, α and β subunits. On the other hand, 11S a hexamer consists of acidic and basic polypeptide linked by disulfide bonds (Mori *et al*. 1981). The 11S proteins have three to four times more sulfur containing amino acids (particularly methionine) than does 7S protein (Beilinson *et al*. 2002). Furthermore, the β subunit of 7S protein is known to be void of methionine and cysteine (Krishnan, 2000). On the other hand, raw soybeans contain a number of allergenic proteins such as Gly m Bd 60 K (α subunit of 7S), Gly m Bd 28 K, Gly m Bd 30 K (Lecin) and protease inhibitors (Kunitz and Bowman–Birk proteins) that can possibly alter the body metabolism of consumers (Krishnan *et al*. 2009; Liener, 1994; Ogawa *et al*. 2000; Norton, 1991). Allergic symptoms to soybean include skin, gastrointestinal, and respiratory reactions and in some cases anaphylaxis (Sicherer and Sampson, 2006). Gly m Bd were recognized by IgE antibodies from soybean sensitive patients with atopic dermatitis (Ogawa, 2000; Krishnan *et al*. 2009). The anti-nutritional Lectin activity is related to its ability to recognize and specifically link to carbohydrates in the membranes of the epithelium cells of the digestive tract. Lectin can produce structural change in the intestinal epithelium and resist gut proteolysis (Pusztai *et al*, 1990). Protease inhibitors, served as storage proteins and as regulator of endogenous proteases, can certainly interfere with protein digestion and consequently exert a negative impact on the utilization of soybean-based protein products (Liener and Kakde 1980). Several protease inhibitors were identified in soybean storage proteins, but most of their activity was thought to be due to Kunitz (KTI) and Bowman–Birk (BBI) proteins, which represents the majority of the bioactive proteins that strongly inhibits trypsin and trypsin-chymotrypsin respectively (Norton, 1991). The inhibitors have been shown to induce pancreatic enzymes, hyper secretion and a fast stimulation of pancreas growth, which is histologically described as pancreatic hypertrophy and hyperplasia (Liener, 1995).

It has been suggested that the levels of 11S and 7S protein and their subunits (Fehr *et al*. 2003; Panthee *et al*. 2004; Taski-Ajdukovic *et al*. 2010) as well as

anti-nutrient proteins (lin *et al*. 2008; Gu *et al*. 2010) vary among genotypes. Thus different cultivars may have dissimilar protein products. The aim of the present work focused on assessing 7S and 11S proteins, their subunits and anti-nutrient protein contents of seed storage soybean proteins of six cultivars which are currently cultivated in Iran, and after that assessing the relationship between these proteins and subunits. Awareness of the relationship between these characters among Iranian varieties could be useful for further and facilitate the ongoing efforts on improving the quality of protein in breeding programs of soybean proteins.

Materials and methods

Plant material

The six adopted Iranian soybean cultivars: Hill, Sahar, 032, 033, DPX and JK, provided by Mazandaran Agriculture Research Center in the north of Iran, were planted in the experimental field of Genetic and Agriculture Biotechnology Institute of Tabarestan (GABIT). After complete growth, plants were evaluated for seed protein content, protein solubility and the composition of seed storage protein.

Measurement of protein content and solubility

About 10 mg seed powder was added to 500 µl of protein extraction buffer (92 mM Tris base pH 8.1, 23 mM CaCl2) and then centrifuged at 14000 rpm for 20 min in 4^{oC}, the supernatant was used for the SDS-PAGE analysis. Protein concentration was determined by Bradford method (Bradford, 1976) with bovine serum albumin (BSA) as standard. The protein content of seed was also determined by Kjeldahl method and the amount of total protein was estimated from percent nitrogen content using a conversion factor of 6.25.

SDS-PAGE analysis

Protein electrophoresis was performed by a vertical slab gel apparatus according to Laemmli, (1970). The staking gel consisted of 6% and the separation gel constituted of 14% polyacrylamid respectively. Amount of 80 µg protein was loaded in the well for all the samples. The molecular weight of the polypeptides was calculated from the standard graph plotted Rf vs. Log. Mol. Wt. of marker proteins electrophoresed (SMO431 Fermentas Co.) along with the samples. To investigate the varietal effect, electrophoresis of the storage proteins in six cultivars was performed in triplicate. Videlicet three aliquots of the same sample were analyzed at the same time. The gels were run simultaneously in the same electrophoretic cell.

Quantifying the composition of seed storage protein profile

Scanned protein gels (Bio-Rad Calibrated Densitometer GS-800) were analyzed with Melanie 6 software. The quantity of each protein band was calculated with the basis of percent volume definition which

is equal to: $\frac{volume}{\sum_{i=1}^{n} Volumes} \times 100$. The volume of a spot is calculated as the volume above the spot outline, which is situated at 75% of the spot height (as measured from the peak of the spot).

Statistical analysis

The assays were carried out using completely randomized design (CRD) with three replications. Statistical analyses were done using SPSS software. The Significant differences between cultivars means were determined by the Duncan's multiple range tests ($P < 0.05$), after the analysis of variance test (ANOVA) for independent samples. Pearson's correlation coefficients were used to determine the degree and significance of association traits.

Results

Seed protein content and solubility

There were significant differences among cultivars for seed protein content and solubility (Table 1). The highest protein content belonged to 033 and the lowest was found for DPX cultivars (42.45 % and 34.90 % respectively). The DPX was also characterized by the highest protein solubility (76.86 mg g^{-1}), whereas the lowest was related to 032 when compared to other cultivars (63.38 mg g^{-1}).

Profiling of seed storage proteins

To identify variants of storage protein subunits in the six adopted Iranian soybean varieties Hill, Sahar, 032, 033, DPX and JK, protein extracts were analyzed by SDS–PAGE. Typical electrophoretic patterns obtained from total proteins are illustrated in Figure 1.

Table 1. Protein content and solubility in seeds of six soybean cultivars.

Genotypes	Protein content (%)	Protein solubility (mg g^{-1})
Hill	41.74 ± 0.07 ^{ab}	70.91 ± 0.52 ^{b}
Sahar	40.94 ± 1.01 ^{ab}	64.80 ± 0.17 ^{c}
033	42.45 ± 0.28 ^{a}	65.58 ± 0.24 ^{c}
DPX	34.90 ± 0.07 ^{c}	76.58 ± 0.46 ^{a}
JK	39.29 ± 0.09 ^{b}	65.58± 0.33 ^{c}
032	41.04 ± 0.26 ^{ab}	63.38 ± 0.06 ^{d}

Mean± Standard Deviation followed by the same letter within the same column are not significantly different at P≤0.05 probability.

The patterns among cultivars were similar, containing the most basic polypeptides. The low level of protein polymorphism could be attributed to conservative nature of the seed protein (Bonfitto *et al*. 1999). However, these patterns could be used as a general biochemical fingerprint for the soybean. The protein banding has the subunits of the major storage proteins, 11S and 7S proteins, including the bulk storage proteins. The 7S subunits separated on SDS–PAGE into three bands of 78, 75 and 47 kDa, corresponding to the ά, α and β subunits of this storage protein respectively. The subunits of 11S separated into five acidic (A) and basic (B) bands. The 11S bands from 34 to 35 kDa correspond to the acidic polypeptide chains A_{1-4}. A_5, the smaller acidic

polypeptide of 11S is designated with 15 kDa.

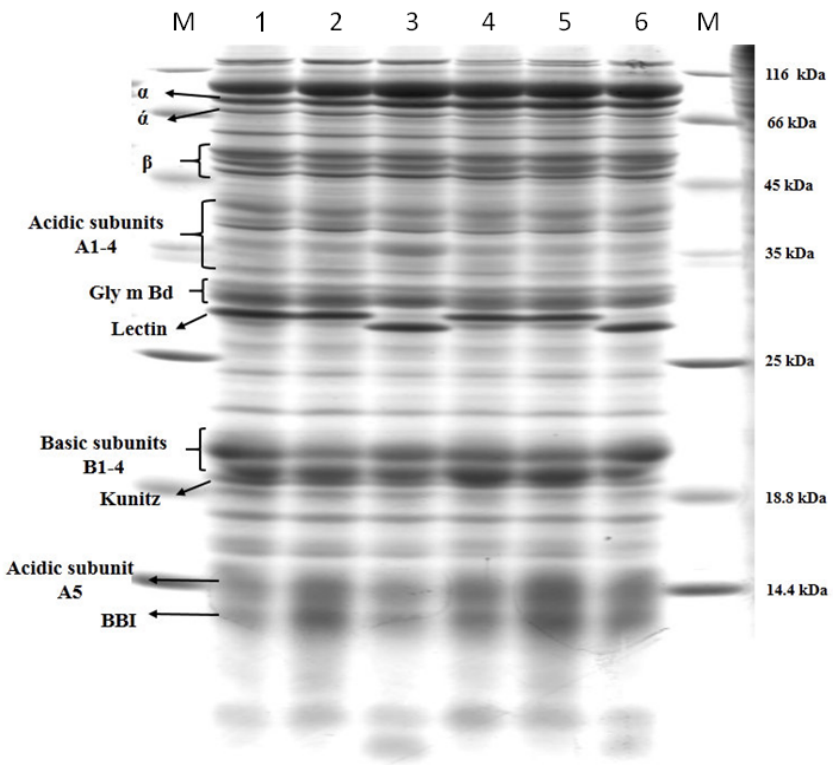

Figure 1- SDS-PAGE analysis of the protein extraction from six soybean cultivars. Lane M, protein molecular marker weight. Lane 1- JK, lane 2, 032. Lane 3, DPX. Lane 4, 033. Lane 5, Sahar. Lane 6, Hill.

The cluster polypeptide that separated on the SDS–PAGE gels from 22 to 23 kDa was the basic polypeptide, designated as B_{1-4}. These results are consistent with Adachi *et al*., (2003) and Maruyama *et al*., (2001) reports. In addition to these components of 11S and 7S subunits, there were four other proteins that were separated as single bands in the SDS–PAGE gel, including the Gly m Bd 28 K, Lectin (Gly m Bd 30 K), Kunitz and Bowman-Birk which are known food allergens of soybeans with 30, 33, 21 and 8 kDa, respectively. The findings of this study are comparable to those reported previously by Yagasaki *et al*. (1997); Yaklich, (2001); Ogawa *et al*. (2000) and Schenk *et al*. (2003) for soybean isogenic lines with different 11S subunit composition.

Quantifying the composition of seed storage protein profile

Densitometry analysis of seed protein profiles was used to quantify the two major storage proteins and their subunits from these cultivars separated on SDS-PAGE (Table 2). The β-conglycinin (7S) content was derived by summation of the original scanned value of ά, α, and β

subunits and the glycinin (11S) content were derived by summation of the acidic and basic components (Figure 1).

Table 2- Comparison of the subunit composition of β-conglycinin (7S) and glycinin (11S) and individual composition of total extractable seed storage proteins by SDS-PAGE and Qualification by densitometry (expressed as percent relative volume of each spot(s)) of six soybean cultivars.

	Genotypes	Hill	Sahar	033	DPX	JK	032
β-Conglycinin	ά	9.08±0.33^{ab}	9.52±0.92^{a}	10.17±0.97^{a}	7.39±0.10^{bc}	6.64±0.02^{c}	6.91±0.55^{c}
	α	3.45±0.05^{b}	4.34±0.01^{a}	4.45±0.02^{a}	3.88±0.20^{ab}	3.66±0.02^{b}	3.82±0.42^{ab}
	β	13.27±0.01^{ab}	11.49±0.07^{bc}	12.59±1.21^{ab}	10.00±0.52^{c}	10.04±0.78^{c}	13.72±0.40^{a}
	7S[1]	25.82±0.38^{a}	25.36±1.00^{a}	27.22±2.15^{a}	21.28±0.62^{bc}	20.35±0.80^{c}	26.46±1.38^{ab}
Glycinin	Total acidic subunits	11.51±0.26^{b}	17.81±1.05^{a}	16.70±1.66^{a}	16.16±0.95^{a}	17.53±0.97^{a}	14.95±0.38^{a}
	Total basic subunits	15.71±0.80^{ab}	14.48±1.13^{bc}	12.74±0.68^{cd}	13.58±0.11^{bcd}	11.60±0.42^{d}	16.79±0.51^{a}
	11S[2]	27.22±1.07^{a}	32.29±2.19^{a}	29.44±2.35^{a}	29.75±0.84^{a}	29.14±1.40^{a}	31.74±0.79^{a}
	11S+7S	53.05±1.46^{bc}	57.65±1.19^{a}	56.66±0.19^{ab}	51.03±1.46^{c}	49.49±2.23^{c}	56.21±0.49^{ab}
	11S/7S	1.05±0.02^{b}	1.28±0.13^{ab}	1.12±0.17^{ab}	1.39±0.01^{a}	1.43±0.01^{a}	1.31±0.11^{ab}
	Gly m Bd 28 K	8.27±0.06^{a}	5.30±0.12^{b}	5.73±0.07^{b}	8.03±0.31^{a}	5.49±0.76^{b}	6.14±1.05^{b}
	Lectin	8.24±0.63^{a}	7.38±0.43^{a}	6.94±0.35^{a}	8.60±1.28^{a}	6.33±0.45^{a}	7.47±0.39^{a}
	KTI[3]	1.17±0.04^{bc}	0.94±0.17^{c}	1.14±0.01^{bc}	1.96±0.19^{ab}	2.28±0.52^{a}	1.99±0.35^{ab}
	BBI[4]	3.36±0.29^{c}	4.01±0.20^{b}	4.00±0.14^{b}	1.33±0.05^{d}	8.83±0.08^{a}	1.12±0.22^{d}

Mean ± Standard Deviation followed by the same letter within the same row are not significantly different at P≤0.05 probability.
1- β-Conglycinin protein, 2- Glycinin protein, 3- Kunitz protein, 4- Bowman-Birk protein.

The values obtained from the densitometer scans were converted to percentage of the total protein in each lane. The obtained results indicated that the 033 and Sahar exhibited the highest concentration of ά (10.17 and 9.52 % respectively) and α subunit (4.45 and 4.34 % respectively) of 7S fraction. Whereas, low concentration of ά subunit were found for 032 and Jk cultivars (6.91 and 6.64 % respectively), and the lowest α subunit was found in Hill and JK (3.45 and 3.66 % respectively). High level of β subunit was found in 032 and the lowest was found in JK and DPX cultivars (10.04 and 10.00 % respectively). Among the cultivars, Hill cultivar flowed by Sahar and 033 were characterized by high concentration of 7S protein (25.82, 25.36 and 27.22 % respectively), while Jk

considered by low level of this protein (20.35 %). All cultivars identified by high level of acidic subunits except for Hill (11.51 %). Whereas, 032 recognized by high concentration of basic subunits (16.79 %) and the lowest was found in JK (11.60 %). No significant difference was found for 11S among the under evaluated cultivars. Nonetheless the Sahar was considered by high concentration of 7S+11S proteins (57.65 %) and the lowest was found in DPX and JK (51.49 and 49.49 %). The DPX and JK cultivars characterized by the high level of 11S/7S ratio (1.39 and 1.43 % respectively), and the lowest was found in Hill cultivar (1.05 %). Among the cultivars, Hill and DPX were considered by significantly high concentration of Gly m Bd 28 K (8.27 and 8.03 % respectively). No significant difference was found between cultivars for Lectin. The JK cultivar was characterized by high concentration of KTI and BBI proteins (2.28 and 8.83 % respectively), whereas low concentration of KTI was found in Sahar (0.94 %) and in DPX and 032 cultivars for BBI (1.33 and 1.12 % respectively).

Correlation analysis

To investigate the relationship between composition of seed storage proteins and their subunits, correlation coefficient analyses were carried out (Table 3). Protein content showed negative correlation with protein solubility and 11S/7S ratio, but had positive correlation with β-subunit and 7S protein. Protein solubility showed no correlation between 7S and 11S proteins and their subunits and 11S/7S ratio, except to 11S+7S and Gly m Bd 28 K. The ά and β subunits of 7S protein had a significant positive correlation with 7S and significant negative correlation with 11S/7S ratio. Also, ά and α subunits of 7S protein showed negative correlation with KTI and Gly m Bd 28 K anti-nutrient proteins. The 7S protein showed positive and negative correlation with 11S+7S and 11S/7S respectively, whereas the 11S protein demonstrates positive correlation with 11S/7S ratio. Only acidic subunits of 11S protein showed positive correlation with 11S and 11S/7S ratio. On the other hand, basic subunits of 11S protein showed negative correlation with the BBI protein. Moreover, 11S/7S ratio showed negative correlation with KTI protein.

Dendrogram studies

Dendrogram of six adopted Iranian soybean cultivars based on seed storage protein composition densitometry using Ward's method showed that cultivars were divided into three clusters (Figure 2). The first clusters consisted of Sahar, 033 and 032 cultivars. Second clusters contained the cultivar JK only, and tertiary cluster included the Hill and DPX cultivars. The lowest genetic distance was recorded between the two cultivars Sahar and 033 and indicated that these cultivars were closely related to each other.

Table 3. Correlation coefficients between β-conglycinin (7S), glycinin (11S) and their subunits and individual components of total extractable seed storage proteins.

Traits	Protein solubility	ά	α	β	7S[1]	Acidic subunits	Basic subunits	11S[2]	11S+7S	11S/7S	Gly m Bd 28 K	Lectin	KTI[3]	BBI[4]
Protein content	-0.66**	0.46	-0.03	0.61**	0.60**	-0.30	0.17	-0.15	0.38	-0.49*	-0.16	-0.05	-0.26	0.15
Protein solubility	1	-0.09	-0.26	-0.37	-0.30	-0.27	-0.09	-0.31	-0.49*	0.02	0.72**	0.51*	0.11	-0.33
ά		1	0.50*	0.42	0.85**	-0.27	-0.17	-0.37	0.49*	-0.81**	-0.12	0.11	-0.65**	-0.13
α			1	0.09	0.47*	0.46	-0.23	0.25	0.59**	-0.18	-0.60**	-0.41	-0.54	-0.10
β				1	0.81**	-0.51*	0.47*	-0.12	0.58*	-0.67**	-0.05	-0.05	-0.47*	-0.39
7S					1	-0.37	0.14	-0.23	0.65**	-0.85**	-0.18	-0.03	-0.70**	-0.31
Acidic subunits						1	-0.26	0.72**	0.25	0.69**	-0.55*	-0.58*	-0.04	0.34
Basic subunits							1	0.47*	0.49*	0.09	0.23	0.13	0.21	-0.61**
11S								1	0.58*	0.70**	-0.33	-0.44	-0.19	-0.12
11S+7S									1	-0.16	-0.42	-0.37	-0.73**	-0.36
11S/7S										1	-0.07	-0.22	0.43	0.19
Gly m Bd 28 K											1	0.54*	0.28	-0.40
Lectin												1	0.22	-0.46
KTI													1	0.15

* and** Significant different at 5% and 1% probability level respectively.

1- β-Conglycinin protein, 2- Glycinin protein, 3- Kunitz protein, 4- Bowman-Birk protein.

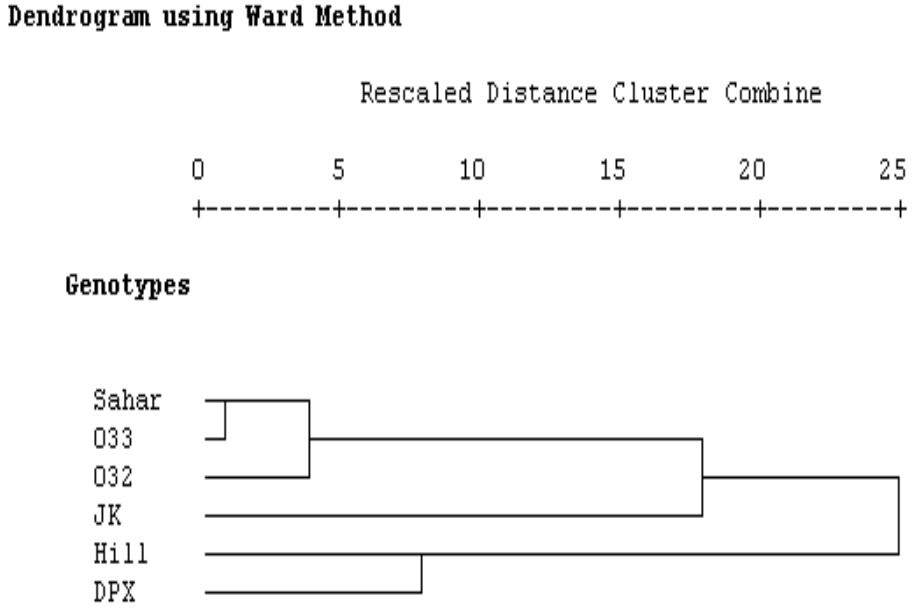

Figure 2. Dendrogram of six soybean cultivars based on seed storage proteins.

On the other hand the highest genetic distance was recorded between the two cultivars Sahar and DPX indicating that these cultivars were genetically distant genotypes.

Discussion

More recently, soybean cultivars have been bred on the seed yield and oil content basis. This regime has resulted in a narrow genetic base that potentially could limit the concurrent improvement in seed yield, protein and oil contents (Kisha *et al*. 1998). Soybean seed storage proteins have a good balance of the essential amino acids required by humans and animals and are mainly used as a source of protein for animal husbandry. With the current increase in meat consumption, the request for protein in animal husbandry has increased. Furthermore, it is relatively inexpensive compared to other protein sources used for livestock. As a result of the extensive use of soybean proteins in the animal industry, humans are also increasingly consuming soybeans and soy products. Earlier studies have shown that the high protein cultivars accumulate higher amounts of both 11S and 7S proteins (Krishnan *et al*. 2007; Yaklich, 2001). A broad range of variability exists among cultivars for all subunit components of 7S, and for both the acidic and basic polypeptide chains of 11S proteins (Table 2). These results are in accord with those of Taski-Ajdukovic *et al*. (2010); Pantee *et al*. (2004); Fehr *et al*. (2003) and Yaklich (2001), who also investigated the

individual components of these proteins in various cultivars.

Today, increasing quality of seed storage proteins is the most important goal in soybean breeding programs. In comparison with meat, soybean proteins are deficient in sulfur containing essential amino acids such as methionine followed by cysteine and possibly threonine (Zarkadas *et al*., 1999). Due to abundance of 7S and 11S, these proteins were the main factors responsible for soybean protein quality (Kitamura, 1995). The ά and α subunits of 7S protein contain traces of methionine and cysteine. In addition, the β subunit of this fraction is known to be void of methionine and cysteine (Krishnan, 2000). Therefore, selection for a low level of the β subunit and high levels of ά and α subunits of 7S protein could help increase the total sulfur containing amino acids. Nevertheless, 11S protein is a better source of sulfur amino acids than 7S due to 3-4.5% more per unit of protein (Beilinson *et al*. 2002; Krishnan, 2000). By comparison the sulfur amino acids of 7S protein account for less than 1% of the amino acid residues (Burton *et al*. 1982). No significant difference was found for 11S among the cultivars under evaluation. A significant and moderate positive correlation was also found between acidic and basic subunits with 11S protein. In this way, Taski-Ajdukovic *et al* (2010) estimated the accumulation of the main seed storage protein subunits, 11S and 7S proteins, among high protein soybean cultivars, to determine how these genotypes preferentially accumulate specific polypeptides in different maturity groups. These authors indicated significant positive correlation between acidic and basic subunits with 11S protein. This indicates that both subunits increase in seeds simultaneously and suggested that selecting the genotypes with high concentration of both the acidic and basic subunits could increase the 11S protein. All cultivars identified by high level of acidic subunits except for Hill, besides 032 were recognized by high concentration of basic subunits.

In a nutritional view the ά and α subunits of 7S protein with an allergenic potential (Krishnan *et al*., 2009) contain much less sulfur amino acids (Kitamura, 1995). Thus, increase in the 11S to 7S ratio should lead to improvement in protein quality (Poysa *et al*. 2006). This ratio of current Iranian soybean cultivars was from 1.05 to 1.43 (Table 2). Reported data revealed that proportion of 11S to 7S ratio in soybean cultivars was varied from 1.26 to 3.40 (Zilic *et al*. 2010; Mujoo *et al*. 2003; Fehr *et al*. 2003). Due to differences in the gelation properties of soybean storage protein fractions, many researchers have attempted to correlate 7S and 11S proteins with tofu quality. Yagasaki *et al*. (1997) indicated that decreasing the 11S to 7S ratios due to the lack of specific subunits, had a negative effect on tofu quality and the food processing properties of soybeans. As reported by Mujoo *et al*. (2003) tofu textural quality can be positively determined using 11S, 11S/7S ratio and

negatively by 7S fraction. Among the cultivars, DPX and JK which were characterized by low concentration of 7S protein and high level of 11S/7S ratio could be a suitable cultivar to gain the higher tofu quality.

On the other hand, Fehr *et al*. (2003) suggested that 11S can be increased at the expense of 7S protein. Ogawa, (1989) also reported a typical inverse relationship between 7S and 11S concentrations. However, Panthee *et al*. (2004) indicated significant positive correlation between 11S with 7S. While, in our study no correlation was found between 7S and 11S proteins. It has been suggested that the 11S, 7S and 11S/7S ratio were influenced by the environment; however no significant differences were expressed among years or locations of these traits (Fehr *et al*. 2003). Therefore it can be the lack of significant differences among the locations which indicated that no site within the test area would be expected consistently different from 11S, 7S and 11S/7S ratio. The difference in the correlation between 7S and 11S in the different studies may be due to the studied cultivars and the number of environments used to evaluate their performance. The results indicated that the importance of genetic improvement on protein quality in soybean breeding program.

In addition, 11S and 7S proteins of the cultivars have a significant positive and negative correlation with the 11S/7S ratio respectively. These results are in agreement with those reported by Fehr *et al*. (2003). However, Pantee *et al*. (2004) reported no relationship between 11S and 11S/7S, but demonstrated significant negative correlation between 7S fraction and 11S/7S ratio. On the other hand, the acidic subunit of glycine protein showed significant positive correlation with 11S/7S ratio. However, Pantee *et al*. (2004) reported positive correlation between basic subunits and 11S/7S. These different results may be due to the genetic differences of cultivars.

More recently, soybean cultivars have been bred to increase seed yield and oil content, while protein meal is mainly used as a source of protein for animal husbandry. A major impediment to increasing soybean protein through selective breeding lies in the inverse relationship between protein content and yield (Helms and Orf, 1998). Nevertheless, it is important to find a balance between protein content and specific protein composition. Taski-Ajdukovic *et al*. (2010) studied forty genotypes of different majority groups of soybean and reported protein content is independent of protein subunits of storage proteins. Fehr *et al*. (2003) also observed no correlation between soybean protein subunits and protein content. These researchers suggested that it is possible to select soybean genotypes for desired protein composition without influencing protein content. However, Yaklich, (2001) and Krishnan *et al*. (2007) showed that high protein cultivars accumulated higher amounts of 11S and 7S proteins. In this study protein content showed positive

correlation whit β-subunit and 7S protein but negative correlation with 11S/7S ratio. Several studies have shown that the accumulation of the β-subunit is promoted by excess application of nitrogen or by sulfur deficiency, while the application of sulfur fertilization increases the synthesis of 11S (Krishnan, 2000). Krishnan *et al*. (2005) have shown that nitrogen application to soybean plants favored the accumulation of β-subunit while decreasing the accumulation of BBI, a protein rich in cysteine. Based on these results, it seems that an inverse relationship exists between protein content and sulfur amino acids content.

Presence of allergenic proteins like α subunit of 7S (Gly m Bd 60 K), Lectin (Gly m Bd 30 K), Gly m Bd 28 K, KTI and BBI makes the deterioration of the soybean protein quality that can possibly alter the body metabolism of consumers (Krishnan *et al*. 2009; Liener, 1994; Ogawa *et al*. 2000; Norton, 1991). An inverse relationship between acidic subunits and Gly m Bd 28 K and Lectin, and also between basic subunits and BBI suggested that genotypes of soybean with high concentration of acidic and basic subunits of 11S protein could decrease these allergenic factors in soybean protein.

In conclusion, results from our study show that, similar seed storage protein pattern exists between six cultivars of soybean which are currently cultivated in Iran. The low level of protein polymorphism could be attributed to conservative nature of the seed protein. However, concentration of 7S and 11S proteins and respective subunits was statistically different among the soybean cultivars. Considering the cultivars, Sahar and 033 with high concentration of ά, α and 7S showed the lowest genetic distance. Moreover, the JK cultivar with low concentration of 7S and basic subunits of 11S proteins and high level of KTI and BBI proteins allocated in one cluster alone. According to the results, JK, which has the lowest concentration of 7S as well as the best 11S/7S protein ratio, could be used as a parent to improve soybean protein quality. On the other hand, 032 with the high level of acidic and basic subunits of 11S fraction and low level of anti-nutrient proteins could also be a suitable cultivar to gain higher seed protein quality. Moreover, the results suggested that development of new genotypes of soybean with high level of acidic subunits of 11S protein which has significant positive correlation with 11S/7S ratio and inverse relationship to some anti-nutrient proteins can be notable in increasing seed storage protein quality in soybean breeding programs.

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References

Adachi, M., Kanamori, J., Masuda, T., Yagasaki, K., Kitamura, K. and Mikami,

B. 2003. Crystal structure of soybean 11S globulin: glycinin A_3B_4 homohexamer. Proc Natl Acad Sci USA, 100: 7395-7400.

Barac, M.B., Sladana, P.S., Snezana, T.J. and Mirjana, B.P. 2004 Soy protein modification. BIBLID, 35:3-16.

Beilinson, V., Chen, Z., Shoemaker, R.C., Fischer, R.L., Goldberg, R.B. and Nielsen, N.C. 2002. Genomic organization of glycinin genes in soybean. Theo and Appl Gen,104: 1132-1140.

Bonfitto, R., Galleschi, L., Macchia, M., Saviozzim, F. and Navari-Izzo, F. 1999. Identification of melon cultivars by gel and capillary electrophoresis. Seed Sci. Tech, 27: 779-83.

Bradford, M.M. 1976. A rapid and sensitive method for quantification of microgram quantities of protein of utilizing the principle dye binding. Analyz Biochem, 72: 680-685.

Burton, J.W., Purcell, A.E. and Walter, W.M. 1982. Methionine concentration in soybean protein from populations selected for increased percent protein. Crop Sci, 22: 430-432.

Fehr, W.R., Hoeck, J.A., Johnson, S.L., Murphy, P.A., Nott, J.D., Padilla, G.I. and Welke, G.A. 2003. Genotype and environment influence on protein components of soybean. Crop Sci. 43:511–514.

Fukushima, D. 1991. Recent progress of soybean protein foods: chemistry, technology and nutrition. Food Review International, 7: 323-351.

Gu, C., Pan, H., Sun, Z. and Qin, G. 2010. Effect of soybean variety on anti-Nutritional factors content and growth performance and nutrients metabolism in rat. Int J Mol Sci, 11: 1048-1056.

Helms, T.C. and Orf, J.H. 1998. Protein, oil, and yield of soybean lines selected for increased protein. Crop Sci, 38: 707-711.

Kisha, T.J., Diers, B.W., Hoyt, J.M. and Sneller, C.H. 1998. Genetic diversity among soybean plant introductions and North American germplasm. Crop Sci. 38: 1669-1680.

Kitamura, K. 1995. Genetic improvement of nutrition and food processing quality in soybean. Japan Agric Res Qua, 29: 1-8.

Krishnan, H.B., Kim, W., Jang, S. and Kerley, M.S. 2009. All three subunits of soybean β-Conglycinin are potential food allergens. J Agric Food Chem, 57: 938-943.

Krishnan, H.B. 2000. Biochemistry and molecular biology of soybean seed storage proteins. Journal of New Seeds, 2: 1-25.

Krishnan, H.B., Bennett, J.O., Kim, W.S., Krishnan, A.H. and Mawhinney, T.P. 2005. Nitrogen lowers the sulfur amino acid content of soybean [Glycine max (L.) Merr.] by regulating the accumulation of Bowman–Birk protease inhibitor. J Agri Food Chem, 53: 6347-6354.

Krishnan, H.B., Savithiry, S.N., Ahmed, A.M. and Randall, L.N. 2007. Identification of Glycinin and β‐conglycinin subunits that contribute to the increased protein content of high‐protein soybean lines. J Agri Food Chem, 55: 1839-1845.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 277: 680-685.

Liener, I.E. and Kakde, M.L. 1980. Protease inhibitors in toxic constituents of plant food stuff. In: I. Liener (ed) 2rd edn, Academic Press, New York, pp 7–71.

Liener, I.E. 1994. Implications of antinutritional components in soyabean foods. Crit Rev Food Sci Nutr. 34: 31-67.

Liener, I.E. 1995. Possible adverse effects of soybean anticarcinogens. J Nutr. 125: 744-750.

Lin, P., Ye, X. and Ng, T. 2008. Purification of melibiose-binding lectins from two cultivars of Chinese black soybeans. Acta Biochim Biophys Sin, 40(12): 1029-1038.

Maruyama, N., Adachi, M., Takahashi, K., Yagasaki, K., Kohno, M. and Takenaka, Y. 2001. Crystal structures of recombinant and native soybean b-conglycinin homotrimers. Europ J Biochem, 268: 3595-3604.

Mori, T., Utsumi, S., Inaba, H., Kitamura, K. and Harada, K. 1981. Differences in subunit composition of glycinin among soybean cultivars. J Agric Food Chem. 29: 20-23.

Mujoo, R., Trinh, D.T. and Perry, K.W. 2003. Characterization of storage proteins in different soybean varieties and their relationship to tofu yield and texture. Food Chem, 82: 265-273.

Norton, G. 1991. Proteinase inhibitors, In FJP D'Mello, CM Duffus, JH Duffus eds, Toxic substances in crop plants, The royal society of chemistry, Cambridge UK, pp 68-106

Ogawa, A., Samoto, M. and Takahashi, K. 2000. Soybean allergens and hypoallergenic soybean products. J Nutr Sci Vitamin, 46(6): 271-279.

Ogawa, T., Tayama, E., Kitamura, K., and Kaizama, N. 1989. Genetic improvement of seed storage proteins using three variant alleles of 7S globulin sub-unit in soybean (*Glycine max* L.). Japan J Breed, 39: 137-147.

Osborne, T.B. and Campbell, G.F. 1898. Proteins of the pea. J Am Chem Soc, 20: 348-362.

Panthee, D.R., Kwanyuen, P., Sams, C.E., West, D.R., Saxton, A.M. and Pantalone, V.R. 2004. Quantitative trait loci for β-conglycinin (7S) and glycinin (11S) fractions of soybean storage protein. JAOCS, 81: 1005-1012.

Poysa, V., Woodrow, L., and Yu, K. 2006. Effect of soy protein subunit composition on tofu quality. Food Res Inter, 39: 309-317.

Pusztai, A., Ewen, S.W.B., Grant, G., Peumans, W.J., van Damme, E.J.M., Rubio, L. and Bardocz, S. 1990. Relationship between survival and binding of plant lectins during small intestinal passage and their effectiveness as growth factors. Digestion, 46 (suppl. 2): 308-316.

Schenk, G., Neidig, M., Zhou, J., Holman, T.R. and Solomon, E.I. 2003. Spectroscopic characterization of soybean lipoxygenase-1 mutants: the role of second coordination sphere residues in the regulation of enzyme activity. Biochemistry, 42: 7294-7302.

Sicherer, S. H. and Sampson, H.A. 2006. Food allergy. J Allergy Clin Immunol, 117: 470-475.

Taski-Ajdukovic, K., Djordjevic, V., Vidic, M. and Vujakovic, M. 2010. Subunit composition of seed storage proteins in high-protein soybean genotypes. Pesq Agrop Brasil, 45: 721-729.

Yagasaki, K., Takagi, T., Sakai, M. and Kitamura, K. 1997. Biochemical characterization of soybean protein consisting of different subunits of glycinin. J Agri Food Chem, 45: 656-660.

Yaklich, R.W. 2001. β-Conglycinin and glycinin in high-protein soybean seeds. J Agri Food Chem, 49: 729-735.

Zarkadas, C.G., Voldeng, H.D., Yu, Z.R. and Choi, V. 1999. Assessment of the protein quality of nine northern adapted yellow and brown seed coated soybean cultivars by amino acid analysis. J Agric Food Chem, 47: 5009-5018.

Zilic, S.M., Barac, M.B., Pesic, M.B, Drinic, S.D.M., Ignjatovic-Micica, D.D. and Srebric, M.B. 2010. Characterization of proteins from kernel of different soybean varieties. J Sci Food Agric, 91: 60-67.

ارزیابی ترکیبات پروتئین‌های ذخیره‌ای دانه در شش رقم سویا سازگار با اقلیم ایران

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چکیده

کیفیت پروتئین‌های دانه سویا اهمیت زیادی در تولید فراورده‌های آن دارد. این کیفیت پروتئینی تحت تاثیر پروتئین‌های ضد تغذیه‌ای و سطوح پائینی از اسیدهای آمینه گوگرددار می‌باشد. در این مطالعه درصد پروتئین دانه و میزان پروتئین‌های قابل حل، در شش رقم از سویا مورد ارزیابی قرار گرفت. همچنین پروتئین‌های ذخیره‌ای دانه نیز با روش SDS-PAGE و آنالیز دنسیتومتری مورد مطالعه قرار گرفتند. نتایج نشان داد که شباهت زیادی بین پروفایل پروتئین‌های دانه این شش رقم از سویا وجو دارد، اما اختلاف معنی‌داری بین میزان تراکم پروتئین‌های بتا-کان‌گلایسینین (۷S) و گلایسنین (۱۱S) و زیر واحدهای پروتئینی آن‌ها وجود داشت. بر طبق این نتایج ارقام ۰۳۳ و DPX به ترتیب بیشترین درصد پروتئین دانه (۴۲/۴۵ ٪) و بیشترین میزان پروتئین قابل حل (۷۶/۵۸ میلی‌گرم بر گرم) را نشان دادند. ارقام DPX و JK نیز بیشترین میزان نسبت ۱۱S/۷S را داشتند (۱/۳۹ و ۱/۴۳ ٪). از طرفی دیگر رقم JK کمترین میزان پروتئین ۷S را به خود اختصاص داده بود (۲۰/۳۵ ٪). همچنین نتایج نشان داد که رابطه منفی و معنی‌داری بین میزان درصد پروتئین و پروتئین‌های قابل حل وجود دارد (r=-۰/۶۶). همبستگی مثبت و معنی‌داری نیز بین زیر واحدهای اسیدی و بازی با پروتئین ۱۱S بدست آمد (به ترتیب ۰/۴۷ و r=۰/۷۲). پروتئین‌های ۱۱S و ۷S نیز به ترتیب همبستگی مثبت و منفی معنی‌داری را با نسبت ۱۱S/۷S نشان دادند (به ترتیب ۰/۸۵- و r=۰/۷۰). از طرفی دیگر زیر واحدهای اسیدی همبستگی مثبت معنی‌داری را با نسبت ۱۱S/۷S و همبستگی منفی و معنی‌داری را با پروتئین‌های ضد تغذیه‌ای نشان داد. این نتایج پیشنهاد می‌کند که توسعه ژنوتیپ‌های جدیدی از سویا با سطوح بالایی از زیر واحدهای اسیدی از بخش ۱۱S می‌تواند بطور قابل توجه‌ای کیفیت پروتئین‌های دانه سویا را افزایش دهد.

کلمات کلیدی: سویا، پروتئین‌های ذخیره‌ای دانه، بتا-کان‌گلایسینین (۷S)، گلایسنین (۷S)، پروتئین‌های ضد تغذیه‌ای.