Plant Omics Journal POJ

POJ 4(4): 228-235 (2011) ISSN:1836-3644

# Salt treatment can change composition of glycinin and β-conglycinin proteins in soybean seed

**Behzad Kaviani[1]*, Seyyedeh Tayyebeh Pourkhalili[1], Reza H. Sajedi[2] and Bita Mosadegh[3]**

**[1]Department of Horticultural Science, Islamic Azad University, Rasht Branch, Rasht, Iran**
**[2] Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran**
**[3]Department of Biology, Faculty of Science, Guilan University, Rasht, Iran**

***Corresponding author: b.kaviani@yahoo.com**

**Abstract**

This study examined the effects of KCl and $NaH_2PO_4$ on quality and quantity changes of soybean seed proteins. These salts affect on protein synthesis. Pot investigations were carried out with soybean [*Glycine max* (L.) Merr. cv. Pershing] grown under various levels of KCl (0, 15, 30, 45, and 60 g) and $NaH_2PO_4$ (0, 0.02, 0.2, and 2 g) both per 100 kg soil. Changes in proteins were determined by SDS-PAGE, Bradford test, measurement of cysteine, total -SH group, and disulfide bonds content. Results showed differences between glycinin and β-conglycinin subunits, amount of total protein and cysteine, also the amount of free sulfur in various levels of KCl and $NaH_2PO_4$. The highest amount of sulfur and acidic subunit of glycinin containing more methionine and cysteine were observed in seeds of plants grown in the soil treated with 0.2 g of $NaH_2PO_4$ with no KCl.

**Keywords:** *Glycine max* (L.) Merril, glycinin, β-conglycinin, protein alteration, sulfur-containing amino acids.

**Introduction**

Soybean is an important source of high quality proteins, fats, vitamins, minerals and other nutrients for both human food and animal feed (Astadi et al., 2008). The economic value of the soybean seed depends on its protein and oil concentration (Brumm and Hurburgh, 1990; Hurburgh, 1994). The final composition of the seed is known to vary by genotype and in response to environmental conditions during seed development (Brummer et al., 1997; Westgate et al., 1999; Yaklich et al., 2002; Fehr et al., 2003; Wilson, 2004; Nichols et al., 2006). A major challenge for the processing industry has been to develop a general model of environmental effects on seed composition to different environmental conditions that produce consistently high quality soybeans. A classical approach to understand the effects of environmental factors on seed composition has been through manipulative experiments (Gibson and Mullen, 1996; Purcell et al., 2004; Pipolo et al., 2004). These experiments evaluate the effects of quantitative and qualitative conditions of environment on seed composition. Soybeans contain approximately 36-38% protein (Brumm and Hurburgh, 2002; Fehr et al., 2003). The two major storage proteins in soybeans are glycinin (11S globulin) and β-conglycinin (7S globulin), which are stored in vacuoles of seed cells, and account for ~40% and ~25% of the total seed protein, respectively (Nielsen et al., 1989; Wilson, 1987; Utsumi et al., 1997). Glycinin is usually a hexametric protein with a *M*r of ~350 KDa, which consists of five subunits ($A_{1a}B_2$, $A_{1b}B_{1b}$, $A_2B_{1a}$, $A_3B_4$ and $A_5A_4B_3$) with each subunit composed of an acidic polypeptide of 37-42 KDa and a basic one of 20 KDa, linked by disulfide bond (Nielsen, 1985; Adachi et al., 2001, 2003). β-conglycinin is a trimeric protein with a *M*r of ~180 KDa (Maruyama et al., 2001) and consists of three subunits, designated ά, α, and β which have a *M*r of 76 KDa, 72 KDa and 53 KDa, respectively. Because glycinin and β-conglycinin greatly impact on the nutritional value and quality of soybean products, these two storage proteins have been studied extensively, and crystal structures have been determined (Adachi et al., 2001, 2003; Maruyama et al., 2001). Historically, soybeans have been bred to increases seed yield (Yaklich, 2001). The storage protein composition is also influenced by plant nutrient availability. The application of some elements especially potassium and phosphorus for improvement of nutritional value in soybean were studied (Imsande, 1997; Walker et al., 1998; Nikolava et al., 2000). The results showed these elements have an important effect on sulfur-containing amino acids (methionine and cysteine) in soybean seed. Compared to meat proteins the storage proteins of soybeans are deficient in sulfur-containing amino acids. Difference between glycinin, β-conglycinin and glycinin:β-conglycinin ratio has been shown to influence the quality of soy food products (Murphy et al., 1997; Cai and Chang, 1999; Tezuka et al., 2000). β-conglycinin, particularly its β subunit is very deficient (less than 1%) in sulfur-containing amino acids, but these amino acids account for 3-4.5% of glycinin (Fukushima, 1991). As a result, the overall methionine content of soybean proteins is only 0.73 to 0.84% on a dry weight basis. Thus, the nutritional quality in soybean can be enhanced by increasing glycinin and decreasing β-conglycinin. Factors stimulating the synthesis of sulfur-containing amino acids are effective on protein quality. Protein synthesis in soybean is highly influenced by some elements, especially phosphorus, potassium and nitrogen (Imsande, 1997). Environmental effects on soybean protein fractions have been reported (Murphy and Ressureccia, 1984; Fehr et al., 2003). A wide range of morphological, physiological and biochemical changes will occur due to alterations in the nutritional conditions of plants particularly in responses to genes and enzymes expression and therefore protein synthesis and activity (Fabre and Planchon, 2000). Significant differences among genotypes and environments have been reported for glycinin, β-conglycinin and glycinin: β-conglycinin ratio (El-Shemy et al., 2000; Fehr et al., 2003;

**Table 1.** The effect of different levels of phosphate dihydrogen sodium ($NaH_2PO_4$) and chloride potassium (KCl) on total protein, total cysteine, total -SH group and total S-S group content of soybean seeds.

| Amount of KCl and $NaH_2PO_4$ (g/100kg) | Total protein (mg/ml) | Total cysteine (μM/mg) | Total SH group (μM/mg) | Total S-S group (μM/mg) |
|---|---|---|---|---|
| 0 + 0 | 0.3599 i | 175.66 efg | 46.97 h | 555.88 j |
| 0 + 0.02 | 0.9624 ab | 166.54 fg | 79.45 efg | 1005.88 efgh |
| 0 + 0.2 | 0.5416 fghi | 484.62 a | 248.41 a | 1535.29 b |
| 0 + 2 | 0.6870 defg | 370.32 b | 143.41 c | 1870.59 a |
| 15 + 0 | 0.5526 fghi | 158.32 fg | 17.29 i | 935.29 gh |
| 15 + 0.02 | 0.8146 bcde | 166.08 fg | 18.05 i | 1447.06 bcd |
| 15 + 0.2 | 1.0385 ab | 112.57 g | 55.93 gh | 705.88 j |
| 15 + 2 | 0.8069 bcde | 185.87 efg | 25.51 i | 1552.94 b |
| 30 + 0 | 0.9819 abcd | 200.68 def | 120.56 d | 944.12 hi |
| 30 + 0.2 | 1.0182 ab | 196.41 ef | 60.65 gh | 1658.82 ab |
| 30 + 2 | 0.9330 abcde | 173.24 efg | 59.05 gh | 1279.42 cde |
| 45 + 0 | 0.7562 cdef | 139.02 fg | 80.69 ef | 529.41 j |
| 45 + 0.02 | 0.7377 cdef | 239.21 cd | 74.75 efg | 1455.88 bc |
| 45 + 0.2 | 1.0681 a | 117.02 g | 61.26 fgh | 714.71 ij |
| 45 + 2 | 0.4622 ghi | 279.97 c | 79.53 ef | 1111.77 efg |
| 60 + 0 | 0.8653 abcde | 129.16 g | 73.07 efg | 582.35 j |
| 60 + 0.02 | 0.7992 bcde | 231.04 cde | 117.38 d | 1076.47 fgh |
| 60 + 0.2 | 0.6312 efgh | 203.84 def | 131.62 cd | 547.06 j |
| 60 + 2 | 0.4453 hi | 283.99 c | 171.71 b | 600 j |

**Table 2.** Analysis of variance (ANOVA) for the effect of different concentration of KCl and $NaH_2PO_4$ on total protein of soybean [*Glycine max* (L.) Merril] seeds.

| Source of variations | df | Means of scuares | F value |
|---|---|---|---|
| Replication | 2 | 0.153 | 33.2732** |
| Factor A | 4 | 0.175 | 37.9352** |
| Factor B | 3 | 0.191 | 41.4432** |
| A×B | 12 | 0.130 | 28.2367** |
| Error | 38 | 0.005 | |

Coefficient of variation: 8.78%, **: Significant at the 0.01 levels

Takahashi et al., 2000). Selection of mutant strains with high levels of glycinin and low levels of β-conglycinin is a long step toward improvement of protein quantity and quality (Biermann et al., 1998; Imsande, 2001). Improvement of protein quality in soybean is also possible by genetic manipulation (Maruyama et al., 2001; Utsumi et al., 2002). The biochemical composition of soybean seeds which can be influenced by growing medium, determine the quality of proteins. Potassium and phosphorus salts play an important role in protein synthesis, enhancing the plant dry weight and seed production, as well as improvement of crop quality. Thus, the purposes of this study were to determine the effect of different levels of KCl and $NaH_2PO_4$ on quantity and quality changes of proteins and their subunits, considered by Bradford test and SDS-PAGE, also on the amount of cysteine, total -SH groups, and disulfide bonds in soybean seed.

## Results

### *Total protein*

The overall results of the total protein contents, total -SH group, disulfide bond, and total cysteine contents are summarized in Table 1. The best concentrations of KCl and $NaH_2PO_4$ for increase of total protein were 30 and 0.2 g/100 kg soil, respectively. The highest amounts of total protein were obtained from seeds of plants grown in the soil treated with 45 g of KCl along with 0.2 g of $NaH_2PO_4$ (1.0681 mg/ml) and 15 g of KCl along with 0.2 g of $NaH_2PO_4$ (1.0385 mg/ml). The lowest amounts of protein were measured from seeds under treatments of control (without salts) (0.3599 mg/ml) and 60 g of KCl along with 2 g of $NaH_2PO_4$ (0.4453 mg/ml) (Table 1, Fig. 1). Analysis of variance indicated that differences among different concentrations of KCl and $NaH_2PO_4$ and interaction of KCl and $NaH_2PO_4$ on total protein contents were significant ($p<0.01$) (Table 2).

### *Total -SH groups*

The highest amounts of total -SH groups were obtained from seeds of plants grown in the soil treated with 0.2 g of $NaH_2PO_4$ without KCl (248.41 μM/mg) and 60 g of KCl along with 2 g of $NaH_2PO_4$ (171.71 μM/mg). The lowest amounts of protein were measured from seeds under treatments of 15 g of KCl without $NaH_2PO_4$ (17.29 μM/mg) and 15 g of KCl along with 0.02 g of $NaH_2PO_4$ (18.05 μM/mg) (Table 1, Fig. 2). Analysis of variance indicated that differences among different concentrations of KCl and $NaH_2PO_4$ and interaction of KCl and $NaH_2PO_4$ on total -SH groups were significant ($p<0.01$)(Table 3).

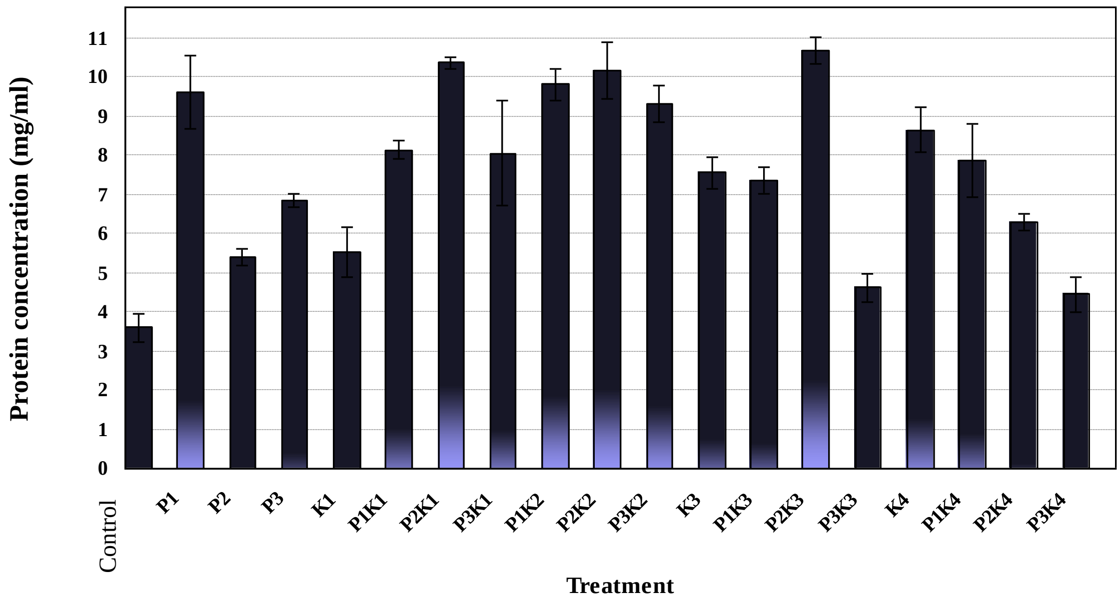


**Fig 1.** The effect of different levels of $NaH_2PO_4$ and KCl on protein concentration of soybean seeds.

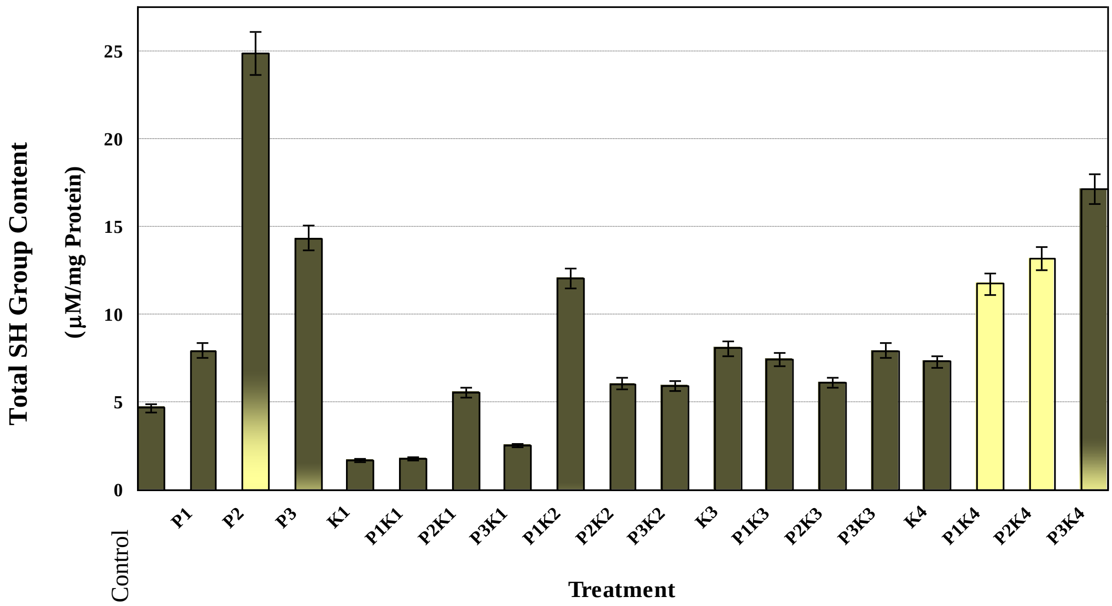


**Fig 2.** The effect of different levels of $NaH_2PO_4$ and KCl on total -SH group content of soybean seeds.

***Disulfide bond contents***

The highest amounts of disulfide bond contents were obtained from seeds of plants grown in the soil treated with 2 g of $NaH_2PO_4$ without KCl (1870.59 µM/mg) and 0.2 g $NaH_2PO_4$ along with 30 g KCl (1658.82 µM/mg). The lowest amounts of protein were obtained from seeds under treatments of 45 g of KCl without $NaH_2PO_4$ (529.41 µM/mg) and control (without salts) (555.88 µM/mg) (Table 1, Fig. 3). Analysis of variance indicated that differences among different concentrations of KCl and $NaH_2PO_4$ and interaction of KCl and $NaH_2PO_4$ on disulfide bond contents were significant ($p<0.01$) (Table 4).

***Total cysteine contents***

The highest amounts of total cysteine contents were obtained from seeds of plants grown in the soil treated with 0.2 g of $NaH_2PO_4$ without KCl (484.62 µM/mg) and 2 g $NaH_2PO_4$ without KCl (370.32 µM/mg). The lowest amounts of protein were obtained from seeds under treatments of 15 g of KCl along with 0.2 g $NaH_2PO_4$ (112.57 µM/mg) and 45 g of KCl along with 0.2 g $NaH_2PO_4$ (117.02 µM/mg) (Table 1, Fig. 4).

**Table 3.** Analysis of variance (ANOVA) for the effect of different concentration of KCl and $NaH_2PO_4$ on -SH groups content of soybean [*Glycine max* (L.) Merril] seeds.

| Source of variations | df | Means of scuares | F value |
|---|---|---|---|
| Replication | 2 | 878.317 | 24.4567** |
| Factor A | 4 | 18324.892 | 510.2556** |
| Factor B | 3 | 4779.350 | 133.0808** |
| A×B | 12 | 6033.336 | 167.9979** |
| Error | 38 | 35.913 | |

Coefficient of variation: 6.83%, **: Significant at the 0.01 levels

**Table 4.** Analysis of variance (ANOVA) for the effect of different concentration of KCl and $NaH_2PO_4$ on S-S bonds content of soybean [*Glycine max* (L.) Merril] seeds.

| Source of variations | df | Means of scuares | F value |
|---|---|---|---|
| Replication | 2 | 90456.950 | 23.6295** |
| Factor A | 4 | 696194.225 | 181.8621** |
| Factor B | 3 | 979302.817 | 255.8167** |
| A×B | 12 | 321869.414 | 84.0798** |
| Error | 38 | 3828.143 | |

Coefficient of variation: 5.84%, **: Significant at the 0.01 levels

**Table 5.** Analysis of variance (ANOVA) for the effect of different concentration of KCl and $NaH_2PO_4$ on cysteine content of soybean [*Glycine max* (L.) Merril] seeds.

| Source of variations | df | Means of scuares | F value |
|---|---|---|---|
| Replication | 2 | 3578.517 | 12.6078** |
| Factor A | 4 | 37642.358 | 132.6218** |
| Factor B | 3 | 22883.133 | 80.6220** |
| A×B | 12 | 20546.703 | 72.3903** |
| Error | 38 | 283.832 | |

Coefficient of variation: 8.02%, **: Significant at the 0.01 levels

Analysis of variance indicated that differences among different concentrations of KCl and $NaH_2PO_4$ and interaction of KCl and $NaH_2PO_4$ on total protein contents were significant ($p<0.01$) (Table 5).

***Electrophoretic pattern of proteins***

The electrophoretic pattern of soybean storage protein subunits, presented in Fig. 5, showed good resolution of the glycinin and β-conglycinin subunits present in the storage proteins. Electrophoretic analysis of proteins showed that molecular weights of polypeptides ranged from 14 to 120 KDa. Changes in thickness of bands revealed significant changes in protein subunits including glycinin and β-conglycinin. A main modification in polypeptide pattern occurred in acidic subunits with M*r* 38 KDa, including the greatest content of s-containing amino acids, when the plants were grown in the soil with 0.2 g of $NaH_2PO_4$ without KCl. The lowest increasing in acidic subunits of glycinin were observed in the control, and plants treated with 15 g of KCl without $NaH_2PO_4$ and 60 g of KCl along with 2 g $NaH_2PO_4$ (Fig. 5). No remarkable decreasing was observed in ά, α, and β subunits of β-conglycinin when the acidic subunits of glycinin were increased (Fig. 5).

**Discussion**

The protein subunits and total protein can be effectively changed by utilization of proper levels of potassium and phosphorus fertilizers. Differences in protein amounts and subunits could be used as an indicator for nutritional conditions. Imsande (1998; 2001) and Fujiwara et al. (1992) found that protein synthesis in soybean was influenced by elements such as phosphorus, potassium, sulfur and nitrogen. The results showed that we can change total protein and protein subunits of soybean seeds toward improvement of nutritional value using suitable levels of potassium and phosphorus fertilizers. Several reports suggest that the relative synthesis of 11S versus 7S protein is preferentially controlled by the availability of sulfur containing amino acids (Thompson and Madison, 1990; Creason et al., 1983). Krishnan et al. (2005) revealed that soybean protein quality could be significantly improved by increasing the concentration of the sulfur-containing amino acids, cysteine and methionine. Soybeans with higher protein content had a significantly lower percentage of sulfur amino acids, while those with lower protein exhibited a higher content of cysteine and methionine. Nitrogen application elevated the protein content but lowered that of the sulfur amino acids. Qualitative and quantitative analysis of soybean seed proteins revealed that phosphorus is the main factor in changing of proteins, their subunits, cysteine, disulfide bonds, and -SH groups. Studies with a trial design similar to that used in our study have given same results of the influence of phosphorus and potassium salts on quantitative and qualitative parameters in soybean protein. Kaviani and Kharabian (2008) evaluated the effects of KCl and $CaHPO_4$ on total protein, glycinin and β-conglycinin subunits of soybean seed and found that these salts can alter the characteristics. These researchers showed that the highest amount of total protein and acidic subunit of glycinin was obtained in seeds of plants grown in the soil treated with 30 g of KCl and 0.02 g of $CaHPO_4$ per 100 kg of soil. Similar to this study, we observed that the best concentrations of KCl and $NaH_2PO_4$ for increase of total protein were 30 and 0.2 g/100 kg soil, respectively. Contrary to this study, the highest amount of sulfur and acidic subunit of glycinin were observed in seeds of plants grown in the soil

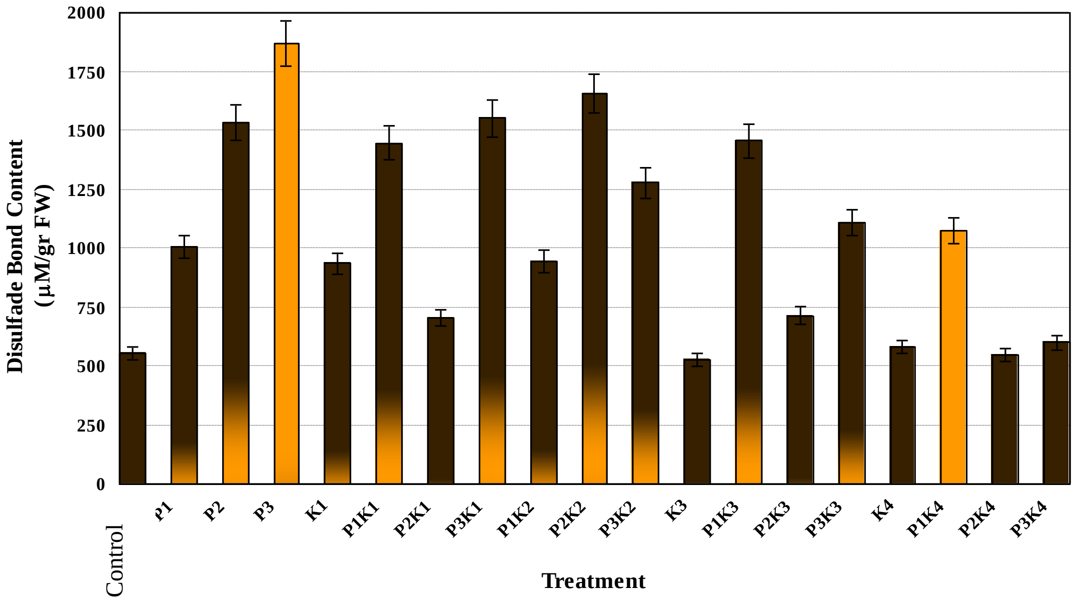


**Fig 3.** The effect of different levels of $NaH_2PO_4$ and KCl on disulfide bond content of soybean seeds.

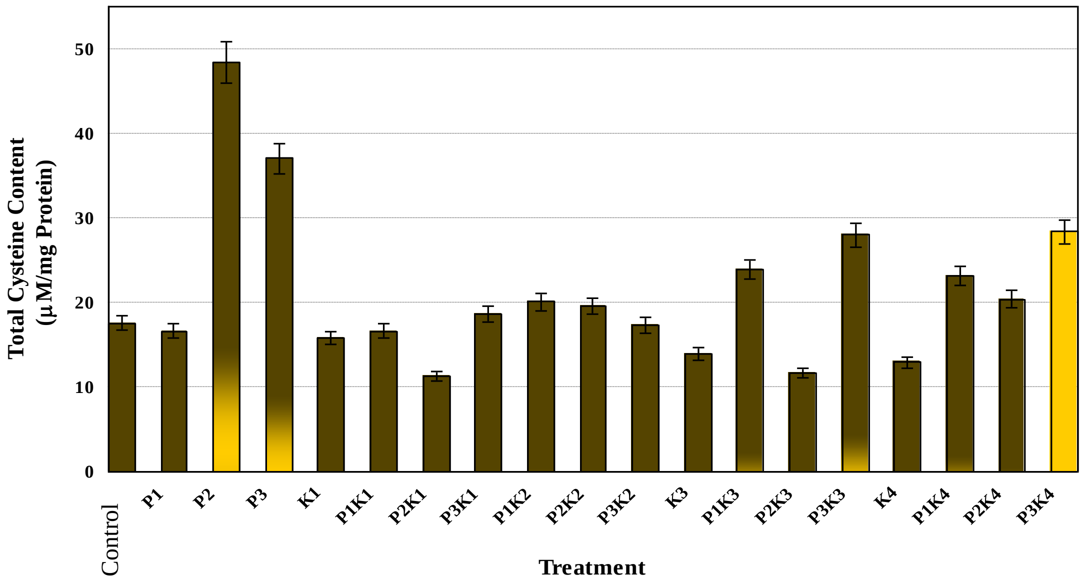


**Fig 4.** The effect of different levels of $NaH_2PO_4$ and KCl on total cysteine content of soybean seeds.

treated with 0.2 g of $NaH_2PO_4$ with no KCl. Current study and studies of Kaviani and Kharabian (2008) showed that the amount of total protein was lowest in control. Kaviani (2007) investigated the effects of KCl and $NaH_2PO_4$ on total protein, glycinin and β-conglycinin of soybean callus and found that the most amounts of total protein and glycinin subunits obtained from calluses grown in the medium containing 30 mM KCl and 1 mM $NaH_2PO_4$. The electrophoresis analysis of our and Kaviani (2007) studies revealed phosphorus salt is the main factor in altering of protein subunits.

In addition, Nikolova et al. (2000) showed that potassium has lesser effect than sulfur on altering soybean seed protein. Many studies have shown that the nutritional stress can alter amounts of soybean storage proteins and their subunits (Sexton et al., 1998; Imsande, 1998; Krishnan et al., 2000; Nikolova et al., 2000; Campo et al., 2009). No straight relation was found between increasing salts in the soil and enhancing total protein in seeds (Table 1). The low concentration of total protein in seeds of the control and seeds produced under the highest levels of salts confirmed this. These results are in agreement with the other results obtained by Kaviani (2007) and Kaviani and Kharabian (2008). Quality of soybean protein is largely determined by its protein composition. This is especially true in the case of β-subunit of β-conglycinin, a protein that is totally devoid of methionine, an essential amino acid (Coates et al., 1985). Eliminating or reducing the β-subunit of β-conglycinin has been suggested as one method of improving the quality of soybean proteins (Wilson, 1987). There is renewed interest in improving the amino acid composition by identifying genotypes that have altered 7S protein composition (Postgate, 1998; Campo et al., 2009). Our primary goal in this report

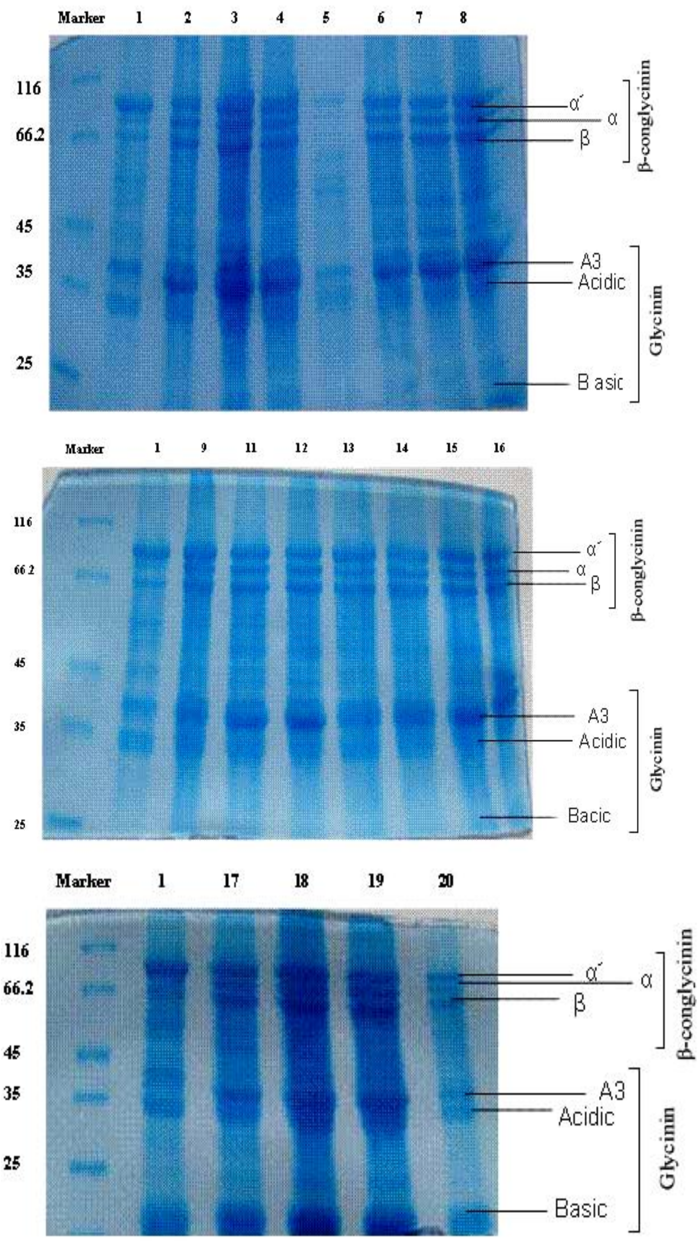


**Fig 5.** One-dimensional SDS-PAGE profile of protein extractions obtained from soybean seeds grown under salts treatments. Gels were stained with Brilliant (Coomassie) Blue R250, and the mass (M*r*) ranges are noted. Treatments numbers are marked with Arabic numerals at the top of the gel. The principal storage polypeptide chains of glycinin and β-conglycinin are identified at the right of the gel.

was to increase of total protein and glycinin along with decrease of β-subunit of β-conglycinin. Here, we were not able to decrease the amount of β-conglycinin subunits in those seeds containing higher glycinin. The results are consistent with the findings of Kaviani (2007) and Kaviani and Kharabian (2008). Our previous studies on the effects of KCl and $NaH_2PO_4$ on soybean callus proteins and KCl and $CaHPO_4$ on soybean seed proteins showed that in treatments, where protein subunits of glycinin were increased, decrease in ά, α, and β subunits of β-conglycinin was observed. Some works have shown that manipulation of β-conglycinin subunits is a difficult and complicated procedure. Many researchers were not able to alter β-conglycinin subunits, even using different levels of nitrogen (Paek et al., 1997) and sulfur (Holowach et al., 1984). Although, Awazuhara et al. (2002) demonstrated that the application of glutathione to immature soybean cotyledons reduced the accumulation of the β-subunit of β-conglycinin and increased the accumulation of glycinin. The study of Carrão-Panizzi et al. (2008) on environmental effects on β-conglycinin and glycinin content in 90 Brazilian soybean cultivars showed that the sowing location had significant effect on β-conglycinin and glycinin protein fractions ratio. Paek et al. (1997) demonstrated that the sulfur-poor β-subunit of 7S protein is more strongly expressed under nitrogen nutritional conditions that promote 7S protein, whereas sulfur containing subunits are influenced less or not at all, thereby deteriorating the 11S/7S ratio. Paek et al. (1997) showed that the seeds developed in a high methionine environment will lose the β-subunit of β-conglycinin.

## Materials & methods

### *Plant material*

Soybean [*Glycine max* (L.) Merril] seeds cv. Pershing were obtained from a breeding institute at Gorgan city in Golestan province located in the northern part of Iran. All measurements were performed on these seeds.

### *Experimental design*

The investigations were carried out in a greenhouse under natural lighting, hydrometric, and temperature conditions. Seeds were sown in pots (18 cm in diameter and 16 cm deep) filled with a 1:1:1 portion of sand: clayey soil: peat mixture. After germination, various levels of potassium (KCl) (0, 15, 30, 45, and 60 g/100 kg soil) ($K_1$, $K_2$, $K_3$, $K_4$, $K_5$) and phosphorus ($NaH_2PO_4$) (0, 0.02, 0.2, and 2 g/100 kg soil) ($P_1$, $P_2$, $P_3$, $P_4$) were added to the soils as treatments (20 treatments). The plants were irrigated daily.

### *Protein extraction*

Freshly harvested seeds were used for protein extraction. Soluble proteins were extracted with Tris-HCl buffer (seed material: buffer, 1:5 w/v) as 100 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 18% sucrose, 40 mM 2-mercaptoethanol, 0.002% Bromophenol Blue, and 2% SDS. The homogenate mixture was centrifuged at 13,000 ×g for 15 min. Supernatant was used for determinations.

### *Total protein measurement*

Total protein was estimated by the dye binding method of Bradford (1976).

### *Total -SH groups, disulfide bonds and cysteine measurement*

Measurement of free -SH groups and disulfide bonds groups were performed by Ellman (1959) using 2, 2-Di-nitrothiobenzoic (DTNB) and Nitrothiosulfobenzoic (NTSB), respectively. The content of cysteine was obtained from sum total of two S-S bonds and -SH groups.

### *SDS-PAGE analysis*

One-dimensional electrophoresis (SDS-PAGE) was performed on vertical slab gels on a 12.5% polyacrylamide gel, and 4% (w/v) stacking gel according to Laemmli (1970). In order to collect soluble proteins from supernatant, 1 ml sample was transferred to 1 ml of incubation medium containing 300 mM Tris-HCl (pH 6.8), 10% sucrose, 2% SDS, 5% 2-mercaptoetanol, and 0.002% Bromophenol Blue and placed for 2 min in boiling water. Clear supernatants obtained after 5 min centrifugation at 5000 ×g were kept for electrophoresis. A 50-100 µL aliquot, containing 50-75 µg protein was loaded onto each of the 10 gel wells. Broad range or pre-stained molecular weight standards were loaded onto the first or last well. Electrophoresis was performed at constant voltage, first at 100 V for 1 h, followed by 125 V until the tracking Bromophenol Blue dye migrated to the bottom of the gel. At the end of the run, the gels were stained with Brilliant (Coomassie) Blue R-250 (0.25% w/v) in methanol-acetic acid (25:10:65 v/v/v), destained with methanol-acetic acid-water (25:7:68 v/v/v), washed overnight with water, and dried between two sheets of Bio Design Gel Wrap drying film.

### *Statistical analysis*

An experiment was carried out using a factorial randomized complete block design (RCBD) with 3 replications. The data processing of the results was carried out by EXCEL package. Analysis of variance was done using MSTATC statistical software under the Windows operating system and means were compared using Tukey's test.

## Conclusion

In conclusion, composition of soybean's seed storage protein is influenced by plant nutrient availability. In the other word, type and composition of soybean growth medium have a high effect on glycinin, β-conglycinin and glycinin: β-conglycinin ratio. Current study confirmed this statement. Application of potassium and phosphorus salts tends to change the total protein contents, total -SH group, disulfide bond, and total cysteine contents of soybean seed.

## References


Adachi M, Kanamori J, Masuda T, Yagasaki K, Kitamura K, Mikami, B (2003) Crystal structure of soybean 11S globulin: glycinin A3B4 homohexamer, Proceedings of the National Academy of Science of the United States of America 100: 7395-7400.

Adachi M, Takenaka Y, Gidamis AB, Mikami B, Utsumi S (2001) Crystal structure of soybean proglycinin A1aB1b homotrimer. J Mol Biol 305: 291-305.

Astadi IR, Astuti M, Santoso U, Nugraheni PS (2008) *In vitro* antioxidant activity of anthocyanins of black soybean seed coat in human low density lipoprotein (LDL). Food Chemistry Doi: 10.1016/j.foodchem.2008.06.034.

Awazuhara M, Kim H, Hayashi H, Chino M, Kim SG, Fujiwara T (2002) Composition of seed storage proteins changed by glutathione treatment of soybean. Biosci Biotechnol Biochem 66: 1751-1754.

Biermann BJ, De Benzie JS, Handelsman J, Thompson JF, Madison JT (1998) Methionine and sulfate increase a Bowma-Birk-Type protease inhibitor and it`s messenger RNA in soybeans. J Agric Food Chem 46: 2858- 2862.

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochem 72: 248–254.

Brumm TJ, Hurburgh CR (1990) Estimating the processed value of soybeans. J Am Oil Chemists Soc 67: 302-307.

Brumm TJ, Hurburgh CR (2002) Quality of the 2002 soybean crop from the United States. St Louis, MO: Am Soybean Association pp 1-14.

Brummer EC, Graef GL, Orf J, Wilcox JR, Shoemaker RC (1997) Mapping QTL for seed protein and oil content in eight soybean populations. Crop Sci 37: 370-378.

Cai T, Cheng K (1999) Processing effect on soybean storage proteins and their relationship with tofu quality. J Agric Food Chem 47: 720-727.

Campo RJ, Aranjo RS, Hungrica M (2009) Molybdenum-enriched soybean seeds enhance N accumulation, seed yield, and seed protein content in Brazil. Field Crops Research 110: 219-224.

Carrão-Panizzi MC, Kwanyuen P, Erhan SZ, Lopes ION (2008) Genetic variation and environmental effects on β-conglycinin and glycinin. Pesq Agropec Bars Brasilia 43 (9): 1105-1114.

Coates JB, Mederiros JS, Thanh VH, Nielsen NC (1985) Characteristics of the subunits of β-conglycinin. Arch Biochem Biophys 243: 59-64.

Creason GL, Holowach LP, Thompson JF, Madison JT (1983) Exogenus methionine depresses level of mRNA for a soybean storage protein. Biochem Biophys Ress Commun 117: 658-662.

Ellman GD (1959) Tissue sulfhydryl groups. Archives of Biochem-is try and Biophysics 82: 70–72.

El-Shemy H, Yamana H, Saneoka H, Fujita K (2000) Phylogenetic comparative analysis of storage proteins structure in some legume seed. Am Biotech Lab 8: 60-62.

Fabre F, Planchon C (2000) Nitrogen nutrition, yield and protein content in soybean. Plant Sci 152: 51-58.

Fehr WR, Hoeck JA, Johnson SL, Murphy PA, Nott JD, Padilla GI, Welke GA (2003) Genotype and enviroment influennce on protein components of soybean. Crop Sci 43: 511-514

Fujiwara TM, Hirai Y, Chino M, Komeda Y, Naito S (1992) Effects of sulfur nutrition on experession of the soybean seed stroge protein genes in transgenic petunia. Plant Physiol 99 (1): 263-268

Fukushima D (1991) Recent progress of soybean protein food chemistry, Tecnology and nutrition. Food Rev Int 7: 323-351.

Gibson LR, Mullen RE (1996) Influence of day and night temperature on soybean seed yield. Crop Sci 36: 98-104.

Holowach LP, Thompson JF, Madison JT (1984) Storage protein composition of soybean cotyledons growth *in vitro* in media of various sulfate concentrations in the presence and absence of exogenous L-methionine. Plant Physiol 74: 584-589.

Hurburgh CR (1994) Long-term soybean composition patterns and their effect on processing. J Am Oil Chemists Soc 71: 1425-1427.

Imsande J (1998) Enhanced nitrogen fixation increases net photosynthesis output and seed yield of hydroponically grown soybean. J Exp Bot 39: 1313-1321.

Imsande J (1997) Nitrogen deficiency during soybean pod fill and increase plant biomass by rapid $N_2$ fixation. Eur J Agr 8: 1-11.

Imsande J (2001) Selection of soybean mutants with increased concentrations of seed methionine and cysteine. Crop Sci 41: 510-515.

Kaviani B (2007) Effect of different levels of potassium chloride and sodium dihydrogen phosphate on the glycinin and β-conglycinin proteins of soybean [*Glycine max* (L.) Merril] callus. Int J Agric Biol 9 (4): 610-612.

Kaviani B, Kharabian A (2008) Improvement of the nutritional value of soybean [*Glycine max* (L.) Merr.] seed with alteration in protein subunits of glycinin (11S globulin) and β-conglycinin (7S globulin). Turk J Biol 32: 91-97.

Krishnan HB, Bennett JO, Kim WS, Krishnan AH, Mawhinney TP (2005) Nitrogen lowers the sulfur amino acid content of soybean [*Glycine max* (L.) Merril] by regulating the accumulation of Bowman-Birk Protease inhibitor. J Agric Food Chem 53: 6347-6354.

Krishnan HB, Jiang G, Krishnan AH, Wiebold WJ (2000) Seed storage protein composition of non-noduling soybean [*Glycine max* (L.) Merr] and its influence on protein quality. Plant Sci 157: 191-199.

Laemmli UK (1970) Cleavage of structural protein during the assembly of the heads of bacteriophage T4. Nature 227: 680-685.

Maruyama N, Adachi M, Utsumi S (2001) Crystal structures of recombinant and native soybean beta-conglycinin beta homotrimers. Eur J Biochem 268: 3595-3604.

Murphy PA, Chen H, Hauck CC, Wilson LA (1997) Soybean protein composition and tofu quality. Food Tech 51: 86-88.

Murphy PA, Ressureccion AP (1984) Varietal and environmental differences in soybean glycinin and β-conglycinin content. J Agric Food Chem 32: 911-915.

Nielsen NC (1985) Structure of soy proteins. In: New Protein Foods, Vol. V, A.M. Altschul and H.LK. Wilcke (eda). Academic press, Orlando, Florida, USA.

Nielsen NC, Dickinson CD, Cho TJ, Thanh VH, Scallon BJ, Fischer RI, Sims TL, Drews GN, Goldbery RB (1989) Characterization of the glycinin family in soybean. Plant Cell 1: 313-328.

Nikolova A, Nedeva D, Tyankova L (2000) Electrophoretic pattens of protein, isolated from soybeam seeds grown under conditions of some mineral deficiency and after different periods of storage. Bulg J Plant Physiol 26: 27-38.

Nichols DM, Glover KD, Carlson SR, Specht JE, Diers BW (2006) Fine mapping of a seed protein QTL on soybean linkage group I and its correlated effects on agronomic traits. Crop Sci 46: 834-839.

Paek NC, Imsande J, Shoemaker RC, Shibles R (1997) Nutritional control of soybean seed storage protein. Crop Sci 37: 489-503.

Pipolo AE, Sinclair TR, Camara GMS (2004) Effects of temperature on oil and protein concentration in soybean seeds cultured *in vitro*. Ann Appl Biol 144: 71-76.

Postgate J (1998) Nitrogen fixation. Cambridge Univ Press, New York, pp 1-112.

Purcell LC, Serraj R, Sinclair TR, De A (2004) Soybean N2 fixation estimates, ureide concentration, and yield responses to drought. Crop Sci 44: 484-492.

Sexton PJ, Naeve SL, Paek NC, Shibles R (1998) Sulfur availability, cotyledon nitrogen:sulfur ratio, and relative abundance of seed storage proteins of soybean. Crop Sci 38: 983-986.

Takahashi M, Hajika M, Matsunaga R, Komatsu K, Obata A, Kanagae R (2000) Breeding soybean variety lacking β-conglycinin by introduction of *Scg* gene from wild soybean. In: International Soybean Processing and Utilization Conference, Tsukuba. Proceedings. Tsukuba: The Japanese Society for Food Science and Technology pp 45-46.

Tezuka M, Tiara H, Igarashi Y, Yagasaki K, Ono T (2000) Properties of tofus and soy milks prepared from soybean having different subunits of glycinin. J Agric Food Chem 48: 1111-1117.

Thompson JF, Madison JT (1990) The effect of sulfate and methionine on legume proteins in H. Renneuberg et al. (eds) SPB Academic Publishing, the Hague, the Netherlands, pp 145-158.

Utsumi S, Matsumura Y, Mori T (1997) Structure-function relationships of soy proteins. In: Damodaran S, Paraf A (eds.) Food Proteins and their Application. New York: Marcel Dekker pp 257-291.

Utsumi S, Maruyama N, Satoh R, Adachi M (2002) Structure function relation of soybean protein revealed by using recombinant systems. Enz Microtechnol 30: 284-288.

Walker DJ, Black CR, Miller AJ (1998) The role of cytosolic potassium and pH in the growth of barley roots. Plant Physiol 118: 957-963.

Westgate ME, Piper E, Bartchelor WD, Hurburgh C (1999) Effects of cultural and environmental conditions during soybean growth on nutritive value of soy products. In: Drackley JK (ed), Soy Animal Nutrition Symposium, Chicago II, 75-89.

Wilson RF (2004) Seed composition. In: Soybeans: Improvement, Production and Users, Third ed. ASA, CSSA, SSSA, Madison, WI.

Wilson RF (2004) Seed metabolism. In: Wilcox JR (ed), Soybeans: Improvement, Production and Uses, No 16, Ame Soc America, USA, pp 643-686.

Yaklich WR (2001) β-conglycinin and glycinin in high-protein soybean seeds. J Agric Food Chem 49: 729-735.

Yaklich RW, Vinyard B, Camp M, Douglass, S (2002) Analysis of protein and oil from soybean northern and southern region uniform tests. Crop Sci 42: 1504-1515.