Turk J Biol
32 (2008) 91-97

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)

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Received: 26.09.2007

Abstract: Protein quality in soybean seeds is strongly influenced by nutritional conditions. Glycinin (11S globulin) and β-conglycinin (7S globulin) are the 2 main proteins in soybean seeds. Protein quality of glycinin (11S) is higher than that of β-conglycinin (7S), due to the presence of higher amounts of s-containing amino acids (methionine and cysteine) in glycinin than β-conglycinin. Of the amino acids in glycinin, 3%-4.5% are methionine and cysteine but these important amino acids are not found in β-conglycinin (especially β-subunit). Pot studies were conducted with soybean [*Glycine max* (L) Merr.] grown under various levels of KCl and $CaHPO_4$ and the effects of salts (fertilizers) on glycinin and β-conglycinin subunits were evaluated. Abundance of glycinin subunits (high quality) and β-conglycinin (poor quality) was determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Quantitative changes in total protein (cytosolic protein) were determined by the Lowry test. Some significant differences were recognized between the measured factors and various treatments. The highest amount of total protein (%) and acidic subunit of glycinin containing more methionine and cysteine was observed 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. A lower amount of protein was observed when no fertilizer (0 P and 0 K) was applied in the growth medium. The current study showed that improvement of soybean nutritional value can be achieved by increasing the content of 11S proteins and decreasing 7S proteins.

Key Words: β-conglycinin, electrophoretic pattern, fertilizer, glycinin, soybean, seed storage protein

Glisinin (11S Globulin) ve β-konglisininli (7S Globulin) Soyafasulyesi [*Glycine max* (L) Merr.] Tohumlarının Besin Değerinin Geliştirilmesi

Özet: Soya fasülyesi tohumunun besin şartları protein kalitesi ile ilişkilidir. Glisinin (11S globulin) ve β-konglisinin (7S globulin) soya fasülyesi tohumunun iki önemli proteinidir. Glisinin (11S) protein kalitesi β-konglisininkinden (7S globulin) daha fazladır. Bunun nedeni glisinin β-konglisininkinden daha fazla amino asite (methionin ve sistein) sahip olmasıdır. Glisininde amino asitlerin % 3-4,5 kadarı metionin ve sisteindir. Fakat bu önemli amaino asitler β-konglisinin de yoktur (özellikle β-altbirimi). Saksı denemelerinde farklı KCl ve $CaHPO_4$ varlığında büyütülen bitkilerde glisinin ve β-konglisininlere tuzun etkisi araştırılmıştır. Glisinin alt birimlerinin (yüksek kalite) ve β-konglisinin (düşük kalite) varlığı sodium dodesil sülfat poliacrilamid jel elektroforesiz (SDS-PAGE) ile çalışılmıştır. Toplam protein miktarındaki değişmeler (stozolik protein) Lowry test yöntemi belirlenmiştir. Ölçülen faktörler ve çeşitli muameleler arasında bazı önemli farklılıklar gözlenmiştir. 30 g KCl ve 0.02 g $CaHPO_4$/100 kg toprakta yetiştirilen bitkilerin tohumlarında en yüksek toplam protein (%) ve glysinin asidik alt birimi fazla metionin ve sistein gözlenmiştir. Büyüme besiyerine gübre konmadığı zaman (0 P ve 0 K) daha az protein miktarı gözlenmiştir. Bu çalışma soya fasülyesinde 11S proteinde artış ve 7S proteinde azalma ile besin değerinin geliştirilmesini göstermektedir.

Anahtar Sözcükler: β-konglisinin, elektroforetik profil, gübre, glisinin, soya fasülyesi, tohum depo proteini

Introduction

Soybean [*Glycine max* (L) Merr.] is a major protein source for humans and other animals. About 90% of soluble proteins in soybean seeds are globulins and more than 70% of globulins are glycinin (G) (11S globulin) and β-conglycinin (βc) (7S globulin). G is relatively rich in s-containing amino acids (methionine and cysteine) (3%-4.5%) and is stored primarily in cotyledons of seeds where it is deposited in protein bodies (1).

G is a hexamer and mainly consists of subunits A3; total A1a, A1b, A2, A4 and acidic and total basic with molecular weight (M*r*) 45 kDa, 38 kDa, and 22 kDa, respectively. βc is a homo- or heterotrimer and consists of 3 subunits, namely α′, α, and β with M*r* 76 kDa, 72 kDa, and 53 kDa, respectively. βc and particularly its β-subunit are poor quality and usually lack s-containing amino acids (2).

The protein composition of soybean seed is not ideal for human nutrition because of the poor content of s-containing amino acids (3). Improvement of soybean nutritional quality can be achieved by increasing the content of 11S proteins and decreasing 7S proteins, in other words increasing the 11S/7S ratio (4,5).

Environmental stress during soybean seed development can alter the chemical composition of seed (6). 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 (7). It has been indicated that protein synthesis in soybean is highly influenced by elements such as phosphorus, potassium, nitrogen, and sulphur (8,9).

Significant differences among genotypes and environments have been reported for G, βc, and G/βc ratio (10,11). Selection of mutant strains with high levels of subunits of 11S and low levels of subunits of 7S is a method for quantity and quality improvement of proteins (12,13). Improvement of protein quality in soybean is possible by genetic manipulation and by entrancing of alien genes into the plant genome (14,15).

In this study we examined the effects of different levels of KCl (K) and $CaHPO_4$ (P) on total protein and G and βc subunits in soybean seeds.

Materials and Methods

Plant material

Mature seeds of soybean [*Glycine max* (L) Merr. cv. Pershing] were harvested from an experimental field at Gorgan in Golestan province in the north of Iran and 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:soil:peat mixture. After germination, various levels of K (0, 15, 30, 45, and 60 g/100 kg soil) and P (0, 0.02, 0.2 and 2 g/100 kg soil) were added to the soils as treatment (20 treatments). The plants were irrigated daily.

Protein extraction

Freshly harvested seeds were used for measurement of total protein and isolation of G and βc subunits. 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 of extracts was evaluated by Lowry test (16).

SDS-PAGE analysis

In order to collect soluble proteins from supernatant, 1-ml samples were transferred to 1 ml of incubation medium containing 300 mM Tris-HCl (pH 6.8), 10% sucrose, 2% SDS, 5% 2-mercaptoethanol, and 0.002% bromphenol blue and placed for 2 min in boiling water. Clear supernatants obtained after 5 min centrifugation at 5000 ×g were kept for electrophoresis. Polypeptides were resolved by electrophoresis of protein in 12.5% PAAG (17). Quantitative differences between protein spectra were evaluated with respect to the intensity of band staining and qualitative differences were estimated based on the number and Rm (relative mobility) values of protein bands. The following protein components were observed as electrophoretic bands after running: α′, α, β, A3 (total A1a, A1b, A2, A4), acidic and total basics. Total A1a, A1b, A2, and A4 were observed as a single band on the gel. Basics were 2 adjacent bands measured separately (18). Electrophoresis patterns were scanned densitometrically.

Statistical analysis

An experiment was carried out using a factorial randomized complete block design with 4 replications. Protein measurements and SDS-PAGE running were also repeated 4 and 2 times, respectively. Analysis of variance (ANOVA) was performed using SPSS and MSTATC software at 5% and 1% probability levels.

Results

Effects of K and P on total protein

The results indicated that total protein was influenced by different levels of K and P. Differences in protein amounts could be used as an indicator for different nutritional conditions.

The highest amounts of total protein were obtained from seeds of plants grown in the soil treated with 30 g of K along with 0.02 g of P, 60 g of K without P, and 15 g of K along with 0.02 g of P (all in 100 kg of soil) in descending order (Table 1). Moreover, the lowest amounts of protein were measured from seeds under treatments of control (blank-without salts), 0.02 g of P without K and 0.2 g of P without K (all in 100 kg of soil) in ascending order (Table 1).

The results of this research showed that 30 g of K and 0.02 g of P had the greatest effect on increasing the total protein amount. Significant differences were found among different levels of salts for total protein.

Analysis of variance indicated that differences among different levels of K and P and interaction of K and P for total protein content were significant ($P < 0.01$) (Table 2).

Table 2 shows that change in potassium level influences protein content regardless of the effect of phosphorus and vice versa. We also found that there is an interaction between these 2 elements, which affects the protein content.

Correlation coefficients for protein-potassium and protein-phosphorus were significant at 89% and 39% of probability, respectively.

Table 1. Effects of different levels of K and P on total protein in soybean seeds.

Treatment no.	KCl (g/100 kg)	$CaHPO_4$ (g/100 kg)	Total protein (mg/ml)
1	0	0	33.26
2	0	0.02	34.59
3	0	0.2	36.01
4	0	2	37.43
5	15	0	43
6	15	0.02	59.62
7	15	0.2	52.71
8	15	2	44.74
9	30	0	40.56
10	30	0.02	68.49
11	30	0.2	44.05
12	30	2	48.34
13	45	0	51.6
14	45	0.02	43.89
15	45	0.2	43.9
16	45	2	50.83
17	60	0	61.86
18	60	0.02	45.07
19	60	0.2	43.39
20	60	2	41.85

Effect of K and P on protein pattern

Figure 1 shows the electrophoresis patterns of the cytosolic proteins extracted from the seeds of soybean grown in soils with different levels of K and P. Electrophoretic analysis of seed cytosolic proteins revealed that molecular masses of polypeptides ranged from 14 to 120 kDa. The electrophoretic pattern of proteins allowed determination of the effect of nutritional conditions on seed storage proteins and relevant subunits. A comparison of electrophoresis bands was performed based on their thickness. Significant changes in seed protein patterns were observed including G (11S globulin) and βc (7S globulin) subunits. The protein subunits can be effectively manipulated by utilization of suitable levels of K and P. A large modification in polypeptide pattern occurred in acidic subunits with M*r* 38 kDa, including the greatest content of s-containing amino acids, when plants were grown in the soils with 30 g

Table 2. Analysis of variance for effect of different levels of K and P on total protein content[a].

Source of variation	Df[b]	Sum of squares	Means of squares	F value	Prob.
Replication	2	51.032	25.516	2.7745	
K	4	1857.069	464.267	50.4813**	0.0000
P	3	431.699	143.9	15.6467**	0.0000
K × P	12	2475.4	206.283	22.4299**	0.0000
Error	2	38	349.479	9.197	0.0000

Coefficient of variation: 6.55%

[a] **: Significant at the 0.01 levels

[b]: Degrees of freedom

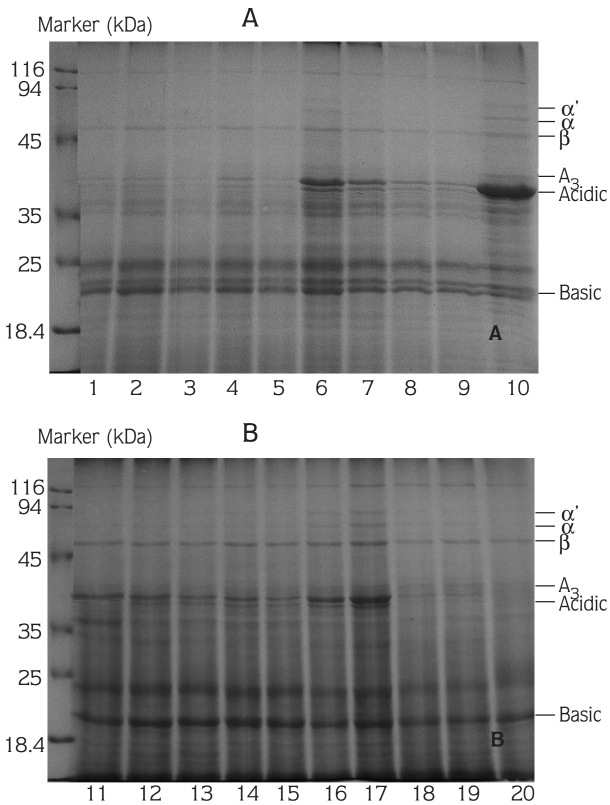

Figure 1. Electrophoretic pattern from cytosolic protein of soybean seeds.
Treatments no. 1 to 10, (A). Treatments no. 11 to 20 (B). The best concentrations of K and P for increasing protein content are 30 g and 0.02 g per 100 kg soil, respectively (Treatment no. 10) (A).

of K and 0.2 g of P, 60 g of K without P, and 15 g of K along with 0.2 g of P, in descending order (Figure 1). The lowest increases in acidic subunits of G were observed in the control (no K or P) and plants treated with 0.2 g of P without K, 15 g of K without P, 60 g of K and 2 g of P, 60 g of K and 0.2 g of P, and 60 g of K plus 0.02 g of P (all in 100 kg of soil), in ascending order. The second most affected proteins were the basic subunits with M*r* 22 kDa. The greatest increases in the basic subunits were observed in plants grown in the soils with 15 g of K and 0.02 g of P and 60 g of K without P. The lowest rates of increase in basic subunits were observed when the levels of K and P were lowest (control, no K or P) or treated with the highest amounts of K and P (60 g of K and 2 g of P).

No remarkable decrease was observed in α′, α and β, subunits of βc (with M*r* 76, 72, and 53 kDa, respectively) when the acidic and basic subunits of G were increased (Figure 1). In other words, α′, α and β, subunits of βc increased in treatments that showed increasing acidic and basic subunits.

Although a slight increase was observed in the rate of A3 subunit in the plants grown in soils with 30 g of K plus 0.02 g of P and 15 g of K plus 0.02 g of P treatments but none of them were significant in all plants.

Electrophoretic analysis and densitometric scans (native PAGE) accurately confirmed the results from protein measurements.

Discussion

Utilization of some elements, especially K and P, for improvement of nutritional value in soybean was evaluated. The results showed these elements have an important effect on s-containing amino acids in soybean seeds.

The examination of the coefficient of correlation revealed that potassium has a greater effect on protein synthesis of soybean seed (probability level of 89%) than phosphorus (probability level of 39%). However, using a suitable strategy to adjust concentrations of these elements to an optimum level will enable us to increase the rate of protein in soybean seeds.

Nikolava et al. (19) showed that deficiency or deprivation of K would cause protein reduction in soybean seeds. Walker et al. (20) have suggested that a decline in cytosolic K below the optimum level leads to protein synthesis reduction and this is the initial cause of growth reduction under K deprivation. Imsande (21) found that protein synthesis in soybean was influenced by elements such as P, K, and N, and the optimum application of these elements increases the storage proteins. Gayler and Sykes (22) also concluded that nutritional stresses can alter amounts of soybean storage protein. The studies on jojoba have also shown that salt stress is generally correlated with changes in the synthesis of proteins (23,24).

No straight relation was found between increasing salts (fertilizers) in the soil and enhancing total protein in seeds (Table 1). The low concentration of total protein in seeds of the control (without salts) and seeds produced under the highest levels of salts confirmed this. The current results can be easily matched with the other results from previous studies (15-17).

Other elements, especially N and S, also affect protein synthesis of soybean seeds. Some researchers have shown that, under N and S deficiency, concentration of total protein is reduced (25,26).

Figure 1 demonstrates that alterations in salt (fertilizers) levels in soil could change the protein subunits of soybean seeds. In this case, no direct correlation or linear equation diagram was found between increasing salts in soil and nutritionally favourable subunits in soybean seeds.

The results showed that we can alter protein subunits of soybean seeds toward improvement of nutritional value using suitable levels of K and P fertilizers.

The highest increasing rate of acidic, basic, and A3 subunits of G (high level of s-containing amino acids) were observed in seeds of plants grown in the soil treated with 30 and 15 g of K and 0.02 g of P. In contrast, the lowest rate of increase was seen in the seeds of plants grown in the soil without P and K (control) and in soil treated with 60 g of K and 2 g of P. The best concentrations of K and P for increasing protein content in soybean seed are 30 g and 0.02 g per 100 kg soil, respectively (Figure 1).

Previous studies in soybean seeds revealed that alterations in K level affect the protein quality of soybean seeds and electrophoretic patterns of seed protein (19).

Our study showed that with increasing acidic subunits of G, which consisted of high levels of s-containing amino acids, no remarkable decreases in βc subunits, especially its β-subunit, with low level of s-containing amino acids were observed. This study also revealed that the highest proportion of acidic subunits of G with M*r* 38 kDa, to β-subunit of βc with M*r* 53 kDa was observed in seeds of plants grown in the soil treated with 30 g of K plus 0.02 g of P per 100 kg of soil.

Numerous studies have shown that manipulation of βc subunits is a difficult and complicated procedure. The vast majority of researchers were not able to alter βc subunits, even using different levels of N (8) and S (27). However, Awazuhara et al. (28) demonstrated that the application of glutathione to immature soybean cotyledons reduced the accumulation of the β-subunit of βc and increased the accumulation of G. Paek et al. (8) demonstrated that the sulphur-poor β-subunit of 7S protein is more strongly expressed under nitrogen nutritional conditions that promote 7S protein, whereas s-containing subunits are influenced less or not at all, thereby deteriorating the 11S/7S ratio. Paek et al. (8) have shown that seeds developed in a high methionine environment will lose the β-subunit of βc. Several reports suggest that the relative synthesis of 11S versus 7S protein is preferentially controlled by the availability of s-containing amino acids.

Cotyledons cultured in a methionine-rich medium do not usually contain β-subunits, despite the unaffected protein concentration (29). When the free methionine concentration drops below a certain level in developing seeds, the synthesis of 11S protein decreases and β-subunit synthesis begins (30). Feeding supplemental methionine to intact plants by stem infusion diminishes the synthesis of the β-subunit (31).

Different cultivars (genotypes) also are significantly different in terms of the G/βc ratio (10).

Conclusion

The type and composition of the soybean's growth medium have a major effect on concentrations of G and βc and G/βc ratio (10,32). This result was verified in the present study. It is suggested that, along with enhancing G subunits, researchers should try to decrease βc subunits. Our study shows that efforts for improvement of soybean seed quality should not be solely focused on protein concentrations.

Acknowledgements

We acknowledge assistance from the following persons: Mr. Amirhossein Darabi and Mr. Ali Rafizadeh for their help with some of the laboratory experiments.

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