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Effect of Different Levels of Potassium Chloride and Sodium Dihydrogen Phosphate on the Glycinin and β-conglycinin Proteins of Soybean [*Glycine max* (L.) Merrill] Callus

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ABSTRACT

The quality of soybean [*Glycine max* (L.) Merrill] food products contain some components of the protein. The objective of this study was to determine the role of KCl and NaH_2PO_4 interactions on the components of glycinin (G) and β-conglycinin (β-c). G (11S) and β - (c7S) are two main proteins (70%) of the soybean. The nutritional quality in soybean can be enhanced by increasing G and decreasing β-c. Soybean calluses were grown in MS' medium containing the different levels of KCl and NaH_2PO_4 for 45 days. Quantitative and qualitative changes in proteins were determined by the Lowry test and SDS-PAGE, respectively. The variance analysis and electrophoretic patterns of proteins showed that there were significant differences between the total protein and G and β-c subunits contents in different levels of KCl and NaH_2PO_4. The results show the possibility of exploring desired levels of these two salts to meet the objective.

Key Words: [*Glycine max* (L.) Merrill]; Glycinin; β - conglycinin

INTRODUCTION

Glycinin (G) (11S globulin) and β - conglycinin (β-c) (7S globulin) are the two main classes of multi-subunit soybean storage proteins, account for approximately 70% of total proteins (Wilson, 1987). The G subunits that can be separated by electrophoresis are A3; total A1a, A1b, A2, A4 and acidic and total basic subunits with M*r* 45-KDa, 38-KDa and 22-KDa, respectively. The components of β-c can be separated into, α´, α and β subunits with M*r* 76-KDa, 72-KDa, and 53-KDa, respectively. Differences in G, β-c and the G:β-c ratio have been shown to influence the quality of soy-food products (Murphy *et al*., 1997; Cai & Chang, 1999; Tezuka *et al*., 2000). β-c, particularly its β subunit is very deficient in sulfur-containing amino acids i.e., methionine (Met) and cysteine (Cys). On the other hand, sulfur-amino acids account for less than 1% of the amino acid residues of β-c. Cys and Met account for 3 - 4.5% amino acid residues of G (Fukushima, 1991). Therefore, increasing this protein would enhance the nutritional quality. Factors stimulating the synthesis of Met and Cys can be affect protein quality. Protein synthesis in soybean is highly influenced by elements such as P, K and N (Imsande, 1997). Significant differences among genotypes and environments have been reported for G, β-c and the G:β-c ratio (El-shemy *et al*., 2000; Fehr *et al*., 2003). Selection of mutant strains with high levels of subunits of 11S and low levels of subunits of 7S also, is a matter for quantity and quality improvement of proteins (Biermann *et al*., 1998; Imsande, 2001). Improvement of protein quality in soybean is possible by genetic manipulation and entrancing foreign genes in plant genome (Maruyama *et al*., 2001; Utsumi *et al*., 2002). The objective of this study was to examine the effects of KCl (K) and NaH_2PO_4 (P) on the total protein and G and β-c subunits in soybean calluses.

MATERIALS AND METHODS

Seeds of soybean were obtained from soybean farms in Gorgan, Iran. Seeds were first disinfected for 2 min in ethanol 70% (v/v), rinsed then sterilized by 2% sodium hypochlorite (w/v) for 15 min. Then, seeds were placed in the water-agar (0.7%) medium, for production of the seedling. After a week, hypocotyls of seedling were divided into sections of 8 to 10 mm and placed in MS medium (Murashige & Skoog, 1962) (basal salt mixture & vitamins suplemented with 3% sucrose, 0.7% Agar-agar, 2 mg L^{-1} 2, 4-D and 0.5 mg L^{-1} kinetin) along with K (0, 15, 30, 45 & 60 mm) and P (0, 0.01, 0.1 & 1 mm) (20 treatments).

For measurement of protein, calluses with 45 days old were used. Proteins were extracted in proportion 3:1 of extraction buffer (0.1 m Tris-Hcl, 0.01 m $MgCl_2$, 18% sucrose, 40 mm 2-mercaptoethanol, 0.002% bromophenol blue and 2% SDS) to callus. The total homogenate was centrifuged (13000 *g* for 15 min). Following centrifugation, the upper phase containing the solution of protein was prepared for measurement of total protein and electrophoresis. Total protein was determined by Lowry test (Lowry *et al*., 1951) and electrophoresis by SDS-PAGE (Laemmli, 1970). The experimental design was "factorial" with "R.C.B." basis design, which was done with unequal repetition. Data were analyzed using "ANOVA" and means were compared at 5% and 1% levels of probability.

RESULTS AND DISCUSSION

Fig. 1 and Table I show the most amounts of protein were obtained from calluses grown in the mediums with treatment no. 12 (68.86 µg), 8 (68.51 µg), 7 (66.82 µg) and 20 (66.26 µg), respectively. In treated no.12, 8 and 20, concentration of P was 1 mm and in no.7 was 0.1 mm. Also, the lowest amount of protein were obtained from calluses grown in the mediums with treatment no.3 (45.36 µg), 13 (46.96 µg), 14 (47.24 µg) and 17 (47.27 µg), respectively. In treatments no.13 and 17 concentration of P was 0 and in no.14 was 0.01 mm. Results showed that in more cases, concentrations of 0.1 and 1 mm P along with difference concentrations K, increase amount of callus protein and 0 and 0.01 mm P along with difference concentrations of K, decrease amount of callus protein.

Analysis of variance showed no significant differences were found between different concentrations of K related to protein synthesis, while differences between concentrations of P and P along with K were significant at 5% and 1% levels of probability, respectively (Table II). Therefore, the most important factor on stimulation of protein synthesis in soybean callus cells was interaction between different concentrations of K and P.

Nikolova *et al*. (2000) showed that K and N had lesser effect than S on altering soybean seed protein. Imsande (2001) found that the protein synthesis in soybean was influenced by elements such as P, K, N and the optimum application of these elements increased storage proteins. Also Gayler and Sykes (1985) concluded that the nutritional stresses can alter amounts of soybean storage protein. Nikolova *et al*. (2000) demonstrated that N, K, B and S deprivation would cause protein reduction in soybean. The present study revealed that there was no direct relationship between increasing salts in growth medium and increasing protein in soybean calluses, which agrees with the findings of Nikolova *et al*. (2000).

The electrophoretic analysis of callus protein revealed P was the main factor in altering of protein subunits. However, the presence of suitable concentrations of K along with P was important for these changes. The comparison of protein bands on gel showed that the amount of protein subunits in all of the callus treatment by 0.1 and 1 mm P were more than those of calluses treated with 0 and 0.01 mm P (Fig. 2 & 3).

The highest change in protein subunits was related to calli resulting from treatment no.12, where acidic subunits of G with M*r* 38-kDa, having the highest content of Met and Cys, showed the greatest increase followed by alkaline subunit, with M*r* 22-kDa. After treatment no.12, the most changes in subunits were seen in treatment no.8 and no.7. The lowest changes of protein subunits were observed in treatments no.3, no.13 and no.14.

In treatments, where protein subunits of G were increased, decrease in α´, α and β subunits of β-c was not observed (Fig. 2 & 3). The results obtained of the electrophoretic analysis confirm the results obtained of Lowry test, exactly. Nikolova *et al*. (2000) on their studies on soybean reported that alteration in N, S and K levels was effective on electrophoretic patterns of seed proteins.

Some researchers did not find changes in β-c subunits, even by using of difference concentrations of N (Paek *et al*., 1997) and S (Holowach *et al*., 1986). Therefore, along with enhance of G subunits one should try to decrease β-c subunits. Efforts for quality improvement of soybean protein should not focus solely on protein concentration. As

Table I. Effects of different levels of salts (KC1 & NaH_2PO_4) on protein content

Treatment No.	KC1 (mm)	NaH_2PO_4 (mm)	Rotein content ($\mu g\ mL^{-1}$)
1	0	0.00	57.04
2	0	0.01	58.08
3	0	0.10	45.36
4	0	1.00	62.9
5	15	0.00	57.53
6	15	0.01	67.33
7	15	0.10	66.82
8	15	1.00	68.51
9	30	0.00	49.46
10	30	0.01	55.4
11	30	0.10	57.51
12	30	1.00	68.86
13	45	0.00	46.96
14	45	0.01	47.24
15	45	0.10	65.82
16	45	1.00	66.2
17	60	0.00	47.27
18	60	0.01	50.81
19	60	0.10	66.24
20	60	1.00	66.26

Table II. Analysis of variance the effect of salts (KCl & NaH_2PO_4) on total protein contents

Treatment	Sum of squars	df	Means of squars	Fs
KC1	4.159	1	4.59	1.599^{NS}
NaH_2PO_4	925.672	2	308.557	9.157*
KC1 NaH_2PO_4 ×	1630.021	3	543.340	203.62**

Fig. 1. Effects of salts (KCl & NaH_2PO_4) on protein content

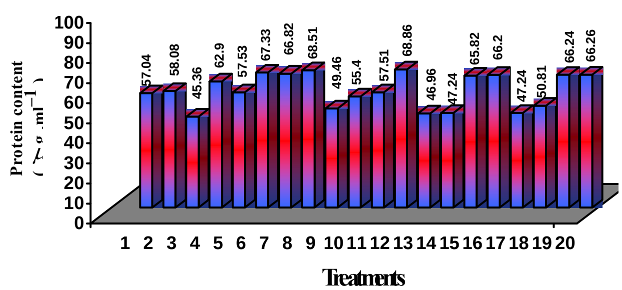


Fig. 2. SDS-PAGE of the subunits of callus protein extracts (Treatment 1-10)

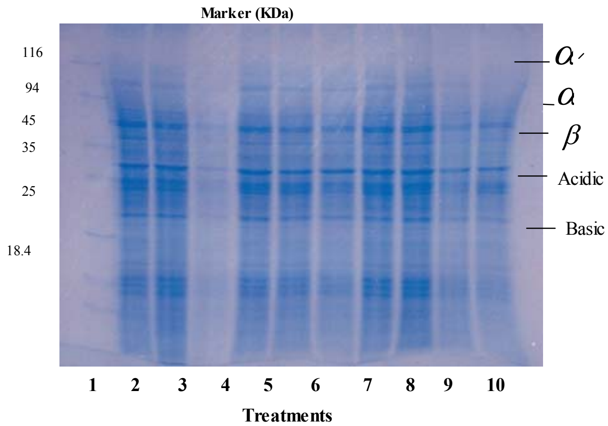
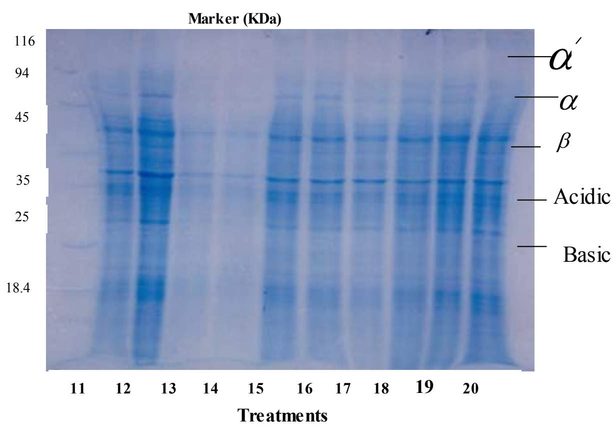


Fig. 3. SDS-PAGE of the subunits of callus protein extracts (Treatment 11–20)



Paek *et al*. (1997) showed increase in protein in soybean grown in high-nitrogen containing medium resulting in the enhanced β-subunit of β-c.

Overall, changes in β-c subunits were fewer. Type and composition of soybean growth medium have a high effect on concentration of G, β-c and the G:β-c ratio. This result has been verified in the present study.

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