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Interrelation between N and S Nutrition on Accumulation of Storage Protein in Soybean Seed

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ABSTRACT

Soybean is an important crop because its seed has very high protein relative to others. The quality of soy protein is limited by the concentration of the sulfur-containing amino acids in the amino acid profile. Among the supply of various forms of 0.4mM sulfur as S nutrition during seed fill, only 0.4mM L-methionine can inhibit β-subunit synthesis completely and produce the highest glycinin-containing seeds. Compared to 0.4mM sulfate control, seeds supplied by 0.4mM L-methionine have lower α-, no β-subunit, and highly increased glycinin without altering total protein concentration. Supply of 0.2mM cystine (0.4mM S) did not affect the accumulative pattern of seed storage protein (SSP) subunits. In the supply of L-methionine, 0.2mM treatment showed higher glycinin in seeds but 0.05mM resulted in lower glycinin than the sulfate control. The relative abundance of α' -subunit was not altered by any N or S nutrition. Under 5mM nitrogen, protein concentration was increased about 3-5% by substituting ammonia for nitrate during seed fill independent of S nutrition. This increase resulted in the only increase of 7S protein, mainly β-subunit. Our data suggest that the regulatory system of SSP genes responds to the balance between N and S assimilates supplied from mother plant, and controls the differential synthesis of their subunits for the maximum protein accumulation in developing soybean seed.

Key words: soybean, seed storage protein, glycinin, β-Conglycinin, sulfate, L-Cystine, L-Methionine, nitrate, urea.

INTRODUCTION

Soybean seeds have been used as a major source of dietary protein for animal feed. The increase of seed protein concentration as well as seed yield has been focused to enhance the crop value of soybean. Currently, the increase of seed protein quality becomes very important for producers and users to increase its crops value because soy protein is lack of sufficient quantities of sulfur amino acids, methionine and cysteine (McVey et al., 1995).

In legume seeds, soybean accumulates about twice as much protein as others, and up to four times as much protein as grains (Larkins, 1981). Soybean seeds contains about 40% of protein in its dry weight (Derbyshire et al., 1976). Storage protein exists about 70% of total protein as a relatively simple subunit structure, which is classified as 7S protein for β -conglycinin and 11S protein for glycinin (Thanh and Shibasaki, 1978). 11S protein is composed of 6 acidic and 5 basic subunits that come from five polypeptides. It can be divided into two protein groups by amino acid homology; Group I ($A_{1a}B_2$, $A_{1b}B_{1b}$, A_2B_{1a}) and Group II ($A_5A_4B_3$, A_3B_4) (Nielsen et al., 1989). 7S protein contains three subunit classes, designated as α(76kD), α' (72kD) and β-subunits (53kD) (Derbyshire et al., 1976). The sulfur amino acid content of 11S protein is approximately 3 to 4.5 % of their amino acid residues that are similar to the content of other high-quality dietary protein (Fukushima, 1991; Nielsen et al., 1989). On the other hand, 7S protein contains less than 1% (Harada et al., 1989; Sebastiani et al., 1990). Among the subunits of β-conglycinin, the mature β-

subunit bears only one cysteine and no methionine residue per unit protein (Coats et al., 1985). Therefore, the β-subunit is primarily responsible for the low sulfur-amino acid content of combined protein of soybean seed.

Most soybean cultivars have about 60% of 11S and 40% of 7S protein in seed storage protein composition (Koshiyama, 1983). To increase the quality of soybean storage protein, the decrease of 7S protein as well as increase of 11S protein without affecting seed storage protein concentration will be a reasonable answer, though it is not accomplished yet. A number of studies have been done to understand genetic regulation of seed storage protein subunits and their accumulations, and to characterize the biochemical processes in developing soybean seed (Fujiwara et al., 1992; Fujiwara and Beachy, 1994; Hirai et al., 1994, 1995; Ladin et al., 1987; Meinke et al., 1981; Naito et al., 1994a, 1994b). We reported recently that their relative accumulation could be controlled differentially by N nutritional condition during seed fill (Paek et al., 1997). The increase of seed storage protein concentration by increasing N nutrition during pod fill results in the only increase of sulfur-poor β-subunit of 7S protein, which causes the decrease of relative abundance of 11S protein though total protein concentration remains constant. N-deficient stress decreases the relative abundance of β-subunits without changing that of α' - and α-subunits (Ohtake et al., 1994; Paek et al., 1997). We speculated that the failure to increase the concentration of 11S protein in parallel with total protein concentration possibly results from the availability of sulfur-amino acids from mother plant into developing seeds. Several reports showed that the relative syntheses of β-conglycinin vs. glycinin (Hirai et al., 1994, 1995) as well as legumin vs. vicillin in pea (Chandler et al., 1983) are controlled by sulfur nutritional condition: 1) soybean cotyledons cultured in a methionine-supplemented medium have no β-subunit accumulation of 7S protein and high concentration of 11S protein without affecting total protein concentration (Creason et al., 1983, 1985; Holowach et al., 1984a, 1984b; 1986; Thompson et al., 1984; Thompson and Madison, 1990), 2) when soybean plants are deprived of sulfur, the initial lag in β-subunit production of β-conglycinin in seed is the same as the sulfur-sufficient controls, but it is succeeded by a rapid increase, and a high rate of production is maintained throughout further development (Gayler and Sykes, 1981, 1985; Fujiwara et al., 1992).

Therefore, N and S nutrition seems to play key roles in controlling the synthesis of protein subunits differentially during seed fill. The objective of the experiment herein was to verify the interrelation between nitrogen and sulfur nutrition for seed storage protein (SSP) synthesis in developing soybean seed.

MATERIALS & METHODS

1. Plant Materials and Treatments

Soybean plants (*Glycine max* [L.] Merrill cv Kenwood) were grown by hydroponic culture in the greenhouse and this experiment was conducted by duplicate trials in two separate rooms at Iowa State University, Ames, USA. The protocols of hydroponic culture were followed as described (Imsande and Ralston, 1981). Solutions were buffered to appropriate pH with 2mM MES (pH 5.7 for nitrate and pH 6.7 for urea), and were changed twice weekly to maintain the nutrient level. Supplemental lightning was provided an additional 200μmole $m^{-2}s^{-1}$ at the plant surface and extended day length to 16hr. The seeds were harvested at R8 stage (Fehr and Caviness, 1977).

2. N and S Nutritional Treatments

Until R4.5 (just before seed development), soybean plants were grown in 5mM KNO_3 and 0.4mM Na_2SO_4 [N/S ratio=12.5] as N and S nutrition to keep sulfur sufficient (SS) condition compared to N concentration (Agrawal and Mishra, 1994). From R4.5 to R8, the plants were divided into two groups by different forms of 5mM N: 5mM KNO_3 (oxidized form) and 2.5mM urea (reduced form: NH_2CONH_2). Different S nutritional conditions were treated in each group with oxidized from (0.4mM Na_2SO_4: control), and reduced forms (0.2mM L-cystine, 0.05mM L-methionine, 0.2mM L-methionine, 0.4mM L-methionine). Duplicate

trials utilized a randomized complete block design and each set of N and S treatments had 4 replications.

3. Protein Extraction, SDS-PAGE and Densitometric Analysis

Ten nature seeds were sampled at random from the whole seeds of each plant. Seed coat was removed and seeds were ground in pestle and mortar for protein extraction. The fine powder was oven-dried at 68° C for 48 hr. Duplicate 0.2g samples were assayed for total protein concentration by micro-Kjedahl (Bremner and Breitenbeck, 1983). Protein extraction from ground tissues and separation of storage protein subunits were followed as described by Paek et al.(1997). Each subunit was identified by their position on the Coommassie-blue-stained gel as indicated(Kitamura, 1995; Paek et al., 1997). The relative abundance of the storage protein fractions was determined with SigmaGel and Peakfit gel analysis software(Jandel Scientific, San Rafael, CA) using images of stained gels obtained with a Scanjet IIcx scanner (Hewlett Packard, USA). Data were subjected to Analysis of Variance for a completely random design.

RESULTS & DISCUSSION

1. Sulfur Nutritional Effects on Accumulation of SSP

Paek et al. (1997) concluded that raising soybean seed protein concentration by increasing plant N assimilation results in decreasing its nutritional quality. The increase results from the only β-subunit of β-conglycinin without increasing the glycinin.

The N and S in the soil exist mostly as oxidized forms such as nitrate and sulfate. Plants must uptake and reduce the oxidized N and S to ammonium and sulfide forms by utilizing their own energy-cost metabolisms for amino acid synthesis, secondary metabolites, etc. In the field, the sufficient S content compared to N was suggested as lower than 16 as N:S ratio in leaf tissue (Agrawal and Mishra, 1994). The higher value causes the sulfur-deficient condition and the lower does the sulfur-sufficient under the given N nutritional state.

To evaluate the effective S forms of increasing S assimilation under 5mM nitrate condition during seed fill, we examined several 0.4mM reduced-S forms under 5mM KNO_3(N/S = 12.5); 1) 0.4mM sodium sulfate as the control [redox value: +6], 2) 0.2mM sodium thiosulfate [+2], 3) 0.4mM sodium sulfide [-2], 4) 0.4mM β-mercaptoethanol [-2], 5) 0.4mM thioacetic acid [-2], 6) 0.4mM thiamine-HCl [-2], 7) 0.4mM thiourea [-2], 8) 0.4mM L-cysteine [-2], 9) 0.2mM L-cystine [-2], 10) 0.4mM L-methionine [-2] (Paek and Yang, 1997). The chemical forms of reduced S treatments(2 to 7) by hydroponic culture show no effects on the relative abundance of SSP subunits compared to the sulfate control. The only S-amino acid forms as sulfur nutrition during seed fill appear to affect the increase of the glycinin and the decrease of β-conglycinin. It speculated that plant should supply the S-amino acids, the final products of S assimilation, into developing soybean seed in order to increase glycinin concentration of total protein.

Among amino acid forms in S nutrition during seed fill (Fig. 1 and 2), more than 0.2mM L-methionine is able to increase the glycinin (11S) concentration in SSP. The 0.2mM L-cystine shows the same relative abundance in their subunits as the sulfate control. It does not show either superior or inferior to the sulfate control. It means that cysteine is not an inducer for increasing the synthesis of 11S protein.

Among different concentration of L-methionine as the sole S source, 0.05mM L-methionine in 5mM N (N/S = 100) is sulfur-deficient level because 11S/7S ratio becomes lower than the sulfate control. More than 0.2mM concentration in 5mM N condition (N/S = 25) becomes the effective level to increase the glycinin concentration in SSP though the N:S ratio is much lower than 16, and 0.4mM concentration of L-methionine (N/S = 12.5) can block completely the sulfur-poor β-subunits of β-conglycinin as reported(Paek and Yang, 1997).

Compared to the sulfate control, seeds supplied by 0.4mM L-methionine during seed fill have unchanged α' -, lower α-, and no β-subunit of 7S protein, and higher 11S protein. The relative abundance of α' -subunit is not altered by any S nutritional conditions.

2. Nitrogen Nutritional Effects on Accumulation of SSP

Paek et al.(1997) showed the increasing protein concentration of soybean seed by the substitution of ammonia for nitrate under the 4mM N concentration during seed fill. N_2 fixation as the sole N source during seed fill also decreased the seed protein concentration due to N deficiency. It means that N nutrition during seed fill is important to increase protein concentration of soybean seed, and N assimilation is still active in plant tissues during seed fill. However, the increasing SSP by the ammonia rather than the nitrate nutrition suggests that the efficiency of N assimilation decrease after R4.5 though the developing seed can afford to accumulate more protein.

In seed TP and SSP in Fig. 1 and 2, the concentrations are totally dependent of N nutritional condition whether sulfur nutrition is oxidized or reduced forms. Increase of seed protein concentration by substitution of nitrate to urea suggests that the increase of N assimilation of mother plant during seed fill would be a key factor for the improvement of seed protein quantity. Under 5mM N condition, protein concentration increases about 3-5% by sub-stituting

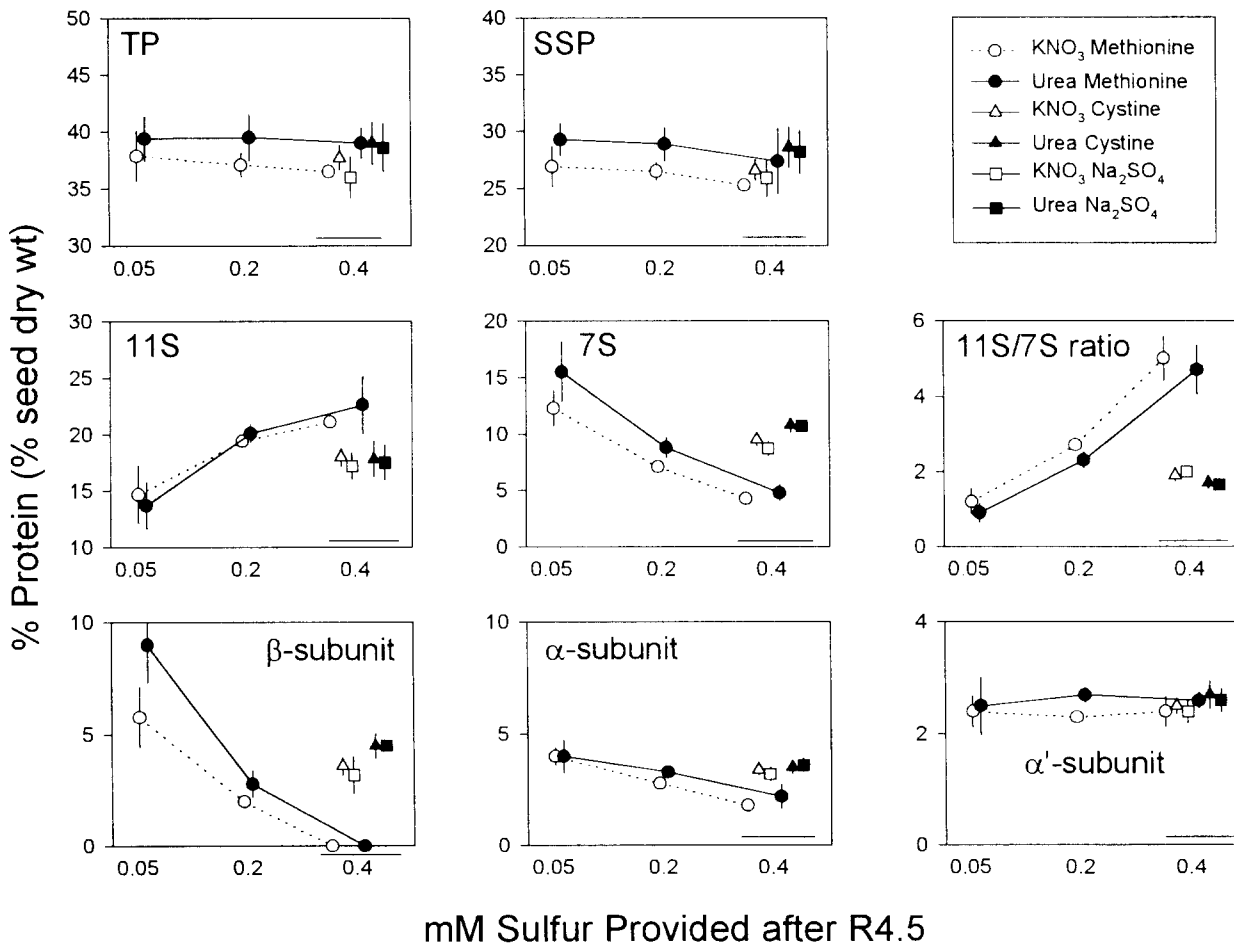

Fig. 1. Effects of N and S source on protein concentration, and 11S/7S ratio in soybean seeds (Trial 1). TP; total protein, SSP; seed storage protein, 11S: glycinin, 7S; β-conglycinin. Plants were grown hydroponically on 5mM KNO_3 and 0.4mM Na_2SO_4 until just before onset of seed filling (R4.5) and then transferred to the respective treatments. Bars indicate standard deviation.

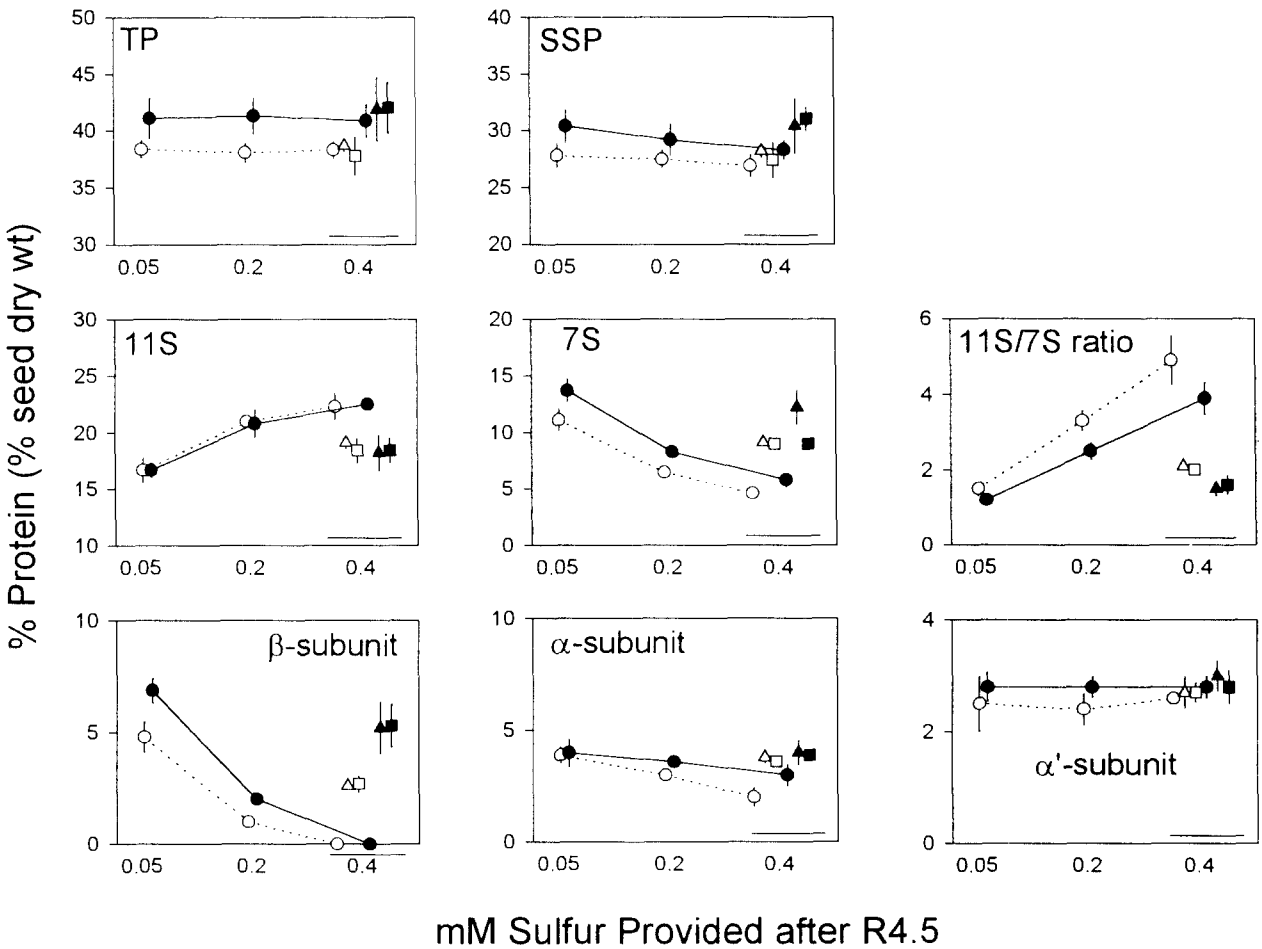

Fig. 2. Effects of N and S source on protein concentration, and 11S/7S ratio in soybean seeds (Trial 2). TP; total protein, SSP; seed storage protein, 11S: glycinin, 7S; β-conglycinin. Plants were grown hydroponically on 5mM KNO_3 and 0.4mM Na_2SO_4 until just before onset of seed filling (R4.5) and then transferred to the respective treatments. Bars indicate standard deviation.

Table 1. N nutritional effects on the percent protein of 7S and 11S protein during seed fill.

Treatment	7S Protein			11S Protein		
	Nitrate	Urea	LSD†	Nitrate	Urea	LSD†
Trial 1						
0.4mM Na_2SO_4	8.9	11.9	**	18.5	18.9	NS
0.2mM L-Cystine	9.0	12.2	**	19.1	18.3	NS
0.05mM Met ǂ	11.1	13.7	**	16.7	16.7	NS
0.2mM Met	6.5	8.3	*	21.0	20.7	NS
0.4mM Met	4.6	5.8	*	22.3	22.4	NS
Trial 2						
0.4mM Na_2SO_4	8.8	10.6	*	17.2	17.6	NS
0.2mM L-Cystine	9.5	10.8	*	18.0	17.7	NS
0.05mM Met	12.3	15.5	**	14.7	13.8	NS
0.2mM Met	7.1	8.8	*	19.4	20.1	NS
0.4mM Met	4.2	4.8	*	21.1	22.5	NS

† Significantly different according to LSD 0.05(*) and 0.01(**). ǂ L-methionine.

ammonia(urea) for nitrate during seed fill inde-pendent of S nutritional condition (Fig. 1 and 2). This increase results in the only increase of 7S protein (Table 1), especially β-subunit as reported by Paek et al. (1997). The concentration of glycinin remains constant in the same S nutritional condition (Fig. 1 and 2, Table 1). Therefore, it appears that the balance of N and S nutrition affects the seed storage protein synthesis differentially.

CONCLUSION

The SSP synthesis of soybean, maybe all legumes, is highly regulated by the N and S nutritional conditions that are undoubtedly supplied from mother plant. Most soybean plants have the self-destructive mechanism to send all the nutritive components to the developing seeds during seed fill. In this concept, the efficiency of assimilation of nitrogen, sulfur and carbon for amino acid synthesis would decrease during seed fill, but may be differently. The regulatory mechanism of SSP genes in developing soybean seed appears to be programmed to control the differential gene expression of SSP subunits in response to the nutrients supplied from mother plant. The first priority of the synthesis of SSP subunits appears to be high S-containing 11S protein. When the L-methionine level among total amino acid pools becomes limited during seed fill, the expression of β-subunit gene is very important to synthesize the low S-containing protein. The β-subunit of β-conglycinin appears to exist as a buffer gene to synthesize more SSP with total amino acid pools containing low S-amino acids. The fluctuation system between the syntheses of 11S and β-subunit of 7S protein contributes coordinately to accumulate the storage protein into seed as more as possible dependent of N and S nutritional condition.

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