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Effects of Sulfur Fertilizer on the Expression of 11S and 7S Seed Storage Proteins of Soybean

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Abstract

The differential response of soybean cultivars with or without sulfur (S) application was observed under field conditions. Plant biomass decreased by sulfur deficiency but the reduction was less in Bragg variety about 26 % relative to the control than other ones over 45%, probably due to less reduction in leaves and pods. The photosynthetic rate of Bragg cultivar was also unaffected by the absence of sulfur application while it depressed in other lines. Soybean cultivars were compared in terms of storage protein, protein quality and biomass production by application of sulfur nutrition. The storage protein concentration tended to decrease without sulfur application in all the cultivars, however the differential response of protein quality only by 11S/ 7S ratio to sulfur nutrition status was observed: For instance, Bragg cultivar had higher biomass and protein production but protein quality decreased at sulfur deficiency. On the other hand, biomass and protein production in other cultivars remained lower at sulfur deficiency but protein quality differed genetically in spite of sulfur nutrition status. These results suggest that the response of soybean to sulfur nutrition is controlled by genotypic difference and sulfur supply status.

Key words: Protein quality, soybean, storage protein, sulfur nutrition.

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Introduction

The deficiency of sulfur amino acids in legume proteins is one of the sever problem due to the increasing of populations. Therefore, this part of the experimental design should be received more attention from scientists to solve the problem. Soybean is an important source of protein for human and livestock production. However, as a sole dietary protein source, soybean seed protein nutrition is constraint with S-containing amino acids such as methionine, and cysteine. Therefore, for instance, increasing the amount of methionine content in the amino acid profile of soybean meal would enhance its nutritional value for consumers and profit for producer.

The nutritional value of soybean meal could be improved by increasing amounts of the S-containing amino acids, methionine and cysteine. Soybean protein is deficient in these amino acids and must be supplemented with other protein sources, or with synthetic methionine, when soybean meal is used as the primary source of protein for humans and for monogastric animals. Glycinin (11S) and β-conglycinin (7S) are the two main classes of seed storage proteins and account for about 70% of total soybean seed protein (Meinke et al. 1981). Glycinin is a well-balanced protein with 3.0 to 4.5% of its amino acid residues consisting of cysteine and methionine (Nielsen et al. 1989; Fukushima 1991), however, β-conglycinin is very deficient in S-containing amino acids (Harada et al. 1989). Paek et al. (1997) concluded that breeding efforts to improve soybean seed protein should not focus entirely on protein concentration. They stated that potentially, soybean protein quality could decline as lines with greater protein concentration are developed.

Several reports suggest that the relative synthesis of 11S and 7S protein is controlled preferentially by the availability of sulfur amino acids: 1) cotyledons cultured in a methionine-rich medium do not contain β-subunit of 11S protein although total protein concentration is unaffected (Holowach et al 1984a,b; Thompson, et al. 1984; Thompson and Madison 1990); 2) as free methionine concentration drops below a certain level in developing seeds, the synthesis of 11S protein decreases and β-subunit synthesis begins (Creason et al. 1983 and 1985); 3) feeding supplemental methionine to intact plants by stem infusion (Giese and Anderson, 1983) or via roots (Peak et al. 1997) diminishes the synthesis of the β-subunit; 4) when soybean plants are deprived of sulfur, the initial lag in β- subunit production in seeds is the same as for the controls but is succeeded by a rapid increase in production; such high rate is maintained throughout further development (Gayler and Sykes 1985). All of the relatively sulfur-rich pea proteins are synthesized in the second half of seed development; thus, the effect of sulfur deficiency appears to be due to a developmental accumulation of proteins for a longer than normal period while the synthesis of the late proteins is suppressed (Higgins 1984). However, this may be coincidental, because in soybeans where the sulfur- containing glycinin and β -conglycinin are developed early, there is a sulfur-poor isomer of β-conglycinin, whose synthesis is restricted to the late stages of the seed development.

Environmental factors such as temperature and plant nutrition may impose another set of controls on storage protein accumulation in addition to the sulfur nutrition and genetic components (Peak et al. 1997; El-Shemy et al. 2000). Hence, sulfur deprivation appears to suppress accumulation of the more sulfur-rich proteins. The β-conglycinin composition varies due to nutritional stress such as S or K deficiency (Gayler and Sykes 1985; El-Shemy et al. 2001).

This experiment was undertaken to examine the effect of the application of S fertilizer on biomass production, protein concentration and quality in soybean cultivars.

Materials and methods

Plant culture and treatment

Soybean (Glycine max L. Merr.) cv. Bragg, Gindaizu, and Fukuyutaka etc. listed in Table (1) were grown from May to October 2000 in the experimental field of Hiroshima University, located in Higashi-Hiroshima City, Japan. N-, P- and K-fertilizer at the rate of 30 kgN ha-1, 90 kgP ha-1, and 149 kgK ha-1 were applied uniformly as a basal dressing to the soil (Granite Regosols) in the form of (NH4)2SO4, superphosphate, K2SO4 for the control plants, and NH4NO3, NaHPO4 and KCl for the S deficient plants, respectively. Soil pH was adjusted to 6.0 with dolomitic calcium carbonate prior to a basal dressing. Nitrogen fertilizer equivalent to 120 kgN ha-1 was applied as a top dressing about 1 month after planting. The experimental design used was a randomized complete block with three replications. Whole plots consisted of 6 rows spaced 80 cm apart with 40 cm between hills. Two seeds were planted per hill and the plants were thinned out to one plant per hill 30 d after planting. Three plants from each plot were individually harvested at the pod filling stage (103 d after planting) and the full maturity, dried in a air-forced draught oven, weighed and ground for N, and S analysis.

Table 1. Soybean cultivars list and country of origin

Cultivar No.	Name (c.v)	Country
1	Tamahomare	Japan
2	Akishirome	Japan
3	Clark	Egypt
4	Sanga	USA
5	Harosoy	USA
6	Bragg	USA
7	Fukuyutaka	Japan
8	Hokuiku 15	Japan
9	Hokuiku 10	Japan
10	Hokuiku 8	Japan

Measurement of photosynthetic rate

Photosynthetic rate was measured by a portable infrared gas analyzer (Model LI-6400 LI-COR Co., Ltd., Lincoln, NE, USA) under natural sunlight. The photosynthetic active radiation was more than 1700 μmolm-2S-1 during measurements. Photosynthetic rates of upper leaves (from top to third leaf on the main stem) of Tamahomare, Bragg and Fukuyutaka cultivars were measured. All the measurements were made with 3 replications.

Measurement of C,N and S

An aliquot of ground leaves and harvested seeds of soybean was used for determination of C, N and S contents. The determination was done made by a CHNS/O analyzer (Perkin Elmer 2400 series II, Wellesley, MA, USA).

Subunit composition

Using ground materials of leaves and harvested seeds of soybean, the following measurement was made. The protein was extracted from 0.5 g defatted seed powder according to the method described by El-Shemy et al. (2000). Twenty five microliters of the solution mixture was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the procedure described by Laemmli (1970), and peptide bands were stained by incubation in Coomassie Brilliant Blue (CBB) G-250 (Sigma Chemical Co., St. Louis, MO, USA). Identification of the components of the major storage proteins was made using a protein marker. The approximate molecular weights of the β-conglycinin subunits corresponded to 76, 72, and 53 kD for the α', α, and β-subunits and those of the glycinin subunits to 45 and 38 kD for the acidic, and 22 kD for the basic subunits, as previously described by Shuttuck- Edidens and Beachy (1985). The peptide bands for β -conglycinin and glycinin were quantified using the scanning gel method (version 1.3.3, PDQUEST MAC, Diversity Database, pdi, Huntington Station, NY, USA). Storage and subunit protein (α', α, and β-subunits, acidic and basic) weights were calculated from those data.

Statistical analysis

All the experiments were conducted with 3 replications. The standard error was calculated according to the methods described by Snedecor (1959).

Results and Discussion

In recent years S-deficiency has become an increasing problem for agriculture resulting in decreased crop quality parameters and yields (McGrath et al. 1996). Appropriate applications of fertilizer can remedy deficiencies in many instances, however, there remain considerable uncertainties regarding timing and type of S-application, which in turn influence the persistence of the S in the soil and the availability to the plant. A common situation is one in which there is a substantial seasonal variation in S available to the plant and, ideally, crops will be engineered to maximize uptake when S is abundant and therefore be better able to tolerate periods of low S-availability. Studies on the mechanisms for controlling sulphate uptake and assimilation suggest approaches for the genetic manipulation of expression of the transporters to engineer crops with improved S-utilization efficiency and S-deficiency stress tolerance (Hawkesford and Smith 1997; Smith et al. 1997).

Biomass production

Prior to identifying targets for genetic manipulation it is necessary to understand the biology of sulphate uptake and assimilation in higher plants, and to have cloned the genes encoding the relevant components. In recent years genes or cDNAs encoding sulphate transporters and enzymes of the assimilatory pathway have been cloned. The reduction was due to more in seed production than vegetative plant parts (Table 2). The seed production response to S deficiency differed among soybean genotypes. The seed weight of Bragg cultivar was about 83% without S application relative to well-supplied S control, while it decreased below 56% in other cultivars (Table 2). The results in the present study are consistent with previous reports showing that soybean biomass production decreases by sulfur deficiency (McGrath et al. 1996; Sexton et al. 1997).

Photosynthetic rate

The higher grain yield in the former cultivar than others under S limitation was due to a higher source activity as found by a higher photosynthetic activity (Table 3) and larger leaf area (data not shown), as well as a higher sink activity as evidenced by greater pods production in spite of lower sulfur content in seeds under S deficiency (Table 2).

On the other hand, photosynthetic rate of Tamahomara

Table 2. Effect of sulfur deficiency on soybean biomass production

Cultivar	No.	Dry weight (g/ plant)			
		Shoots	Roots	Pods	Whole plant
Control	1	26.43±1.2	3.93±1.5	38.27±1.4	68.63±2.0
	6	44.96±1.7	13.38±0.4	34.07±1.7	92.41±3.2
	7	36.50±1.9	6.37±1.2	30.81±1.2	73.68±2.7
-S	1	11.25±0.6	0.99±0.2	17.49±1.1	29.73±1.1
	6	41.55±1.5	2.51±0.4	33.04±1.2	77.1±2.5
	7	21.81±0.8	4.95±1.2	14.78±0.3	41.54±1.9

Table 3. Effect of sulfur deficiency on soybean leaf photosynthetic rate

Cultivar No.		Photosynthetic rate	Cond	Ci	LSD (0.05)
Control	1	17.6	0.271	233	1.61
	6	15.4	0.151	170	0.88
	7	19.6	0.260	207	1.7
- S	1	15.51	0.321	257	0.739
	6	18.85	0.262	215	1.364
	7	16.99	0.259	231	0.65

Photosynthetic rate (μmol CO_2 m^{-2} S^{-1})
Cond: Stomatal conductance (mol H2O m^{-2} S^{-1})
Ci: Leaf internal CO_2 concentration (μL CO_2 L^{-1})
LSD (0.05): Least significant difference at 0.05 level.

Table 4. Effect of sulfur deficiency on nitrogen and sulfur content in matured seeds.

Cultivar No.	Nitrogen (mg/gDW)		Sulfur (mg/gDW)	
	Control	- S	Control	- S
1	59±1.6	58.6±1.7	4.8±0.4	4±1.1
2	67.1±1.4	66.6±1.4	4.7±1.5	4.2±0.8
3	68.9±1.2	65.7±1.4	4.7±0.8	3.9±0.4
4	69.7±1.5	64.3±1.1	5.3±1.2	4.4±0.3
5	60.3±1.5	63.3±1.2	4.7±1.2	3.9±1.2
6	67±1.7	58.2±1.8	4±1.2	3.3±1.4
7	74.8±1.9	68±1.3	4.4±0.3	4.1±0.6
8	64.4±1.4	64±1.0	3.8±1.6	3.8±1.2
9	64.7±1.4	57.8±2.2	4.3±1.1	3.6±1.1
10	62.9±2.0	62.7±2.1	4.1±1.1	3.3±0.6

and Fukuytaka cultivars were decreased under sulfur application compared with Bragg cultivar (Table 3). These results suggest that a soybean Bragg cultivar takes advantage for less adverse affection on seed production by lowering S fetilization. The results indicated that tolerance of Bragg soybean cultivar to sulfur deficiency in terms of biomass production and photosynthesis is due to tolerance of not only source activity in leaves but also sink activities in pods. Our results using some soybean cultivars colloborate with a report showing limitation of photosynthetic rate in soybean under S deficiency (Sexton et al. 1997) in which the decline of photosynthesis is due to limiting the amount of Rubisco content owing to reduction of methionine and cysteine availability (Anderson 1990). However, Sexton et al. (1997) postulated that a limitation on sink strength also could be involved in the decline of the soybean photosynthesis by S deficiency. A sink limitation leading to increased carbohydrate levels in source leaves, which occurred in our study (data not shown), also might contribute to a decrease in Rubisco fraction by causing Rubisco synthesis to be down regulated (Krapp and Stitt1995). Ferreira and Teixeira (1992) working with common duck weed observed that S deficiency caused preferential degradation of Rubisco protein.

Sulfur and nitrogen contents

N content ranged from 59~75 mg/g DW in seeds and was higher in Fukuyutaka, followed by Sanga and lowest in Tamahomare (Table 4). It tended to decrease by S deficiency treatment except for a reverse trend in Harosoy and a slight changed in Hokuiku15 cultivar. S content of control plant ranged from 3.8~5.3 mg/ g DW. S deficiency had significantly reduced the S content in seeds of cultivars except for Hokuiku8 (Tables 4, 5). Matured soybean in the field was fertilized using sulfur growing well without any problems, and meanwhile the other soybean doesn't received the sulfur showed weakness growth and the leafs were turned from green to yellow color due to the lack of sulfur fertilizer.

Table 5. Effect of sulfur deficiency on nitrogen and sulfur content in leaves

Cultivar No.	Nitrogen (mg/gDW)		Sulfur (mg/gDW)	
	Control	- S	Control	- S
1	33.2±1.2	25±0.9	3.3±0.3	1.7±0.1
2	30.4±1.1	27.3±1.2	2.5±0.1	0.8±0.1
3	37.9±1.1	40.8±1.5	1.9±0.1	5±0.2
4	38.6±0.6	29.6±0.5	1.3±0.1	1.2±0.1
5	26.4±0.8	24.4±0.4	0.2±0.01	1.1±0.1
6	42.2±1.1	31.1±1.1	2.1±0.2	0.6±0.1
7	48.4±1.5	35.2±1.3	1.2±0.1	0.5±0.1
8	33.6±1.2	25.2±0.3	0.5±0.01	1.1±0.1
9	30.9±0.5	29.1±0.8	3.6±0.2	1.4±0.1
10	33±0.8	31.7±1.2	2.1±0.1	0.6±0.01

Storage proteins production

In order to evaluate weather the sulfur was affecting on the protein biosynthesis the total protein from leaves and matured seeds was extracted and 25 μg loaded on SDS-PAGE analysis. The results showed that, in the case of leaves there are a slight differences in the components of 7S and 11S proteins and this is caused by sulfur defect in some soybean lines (Figure 1). Redistribution of S from mature leaves is not enhanced by S stress, as shown in studies with barley (Adiputra and Anderson 1995). The insoluble S in mature leaves decreased by about 50% after 7 d when the sulfate supply was discontinued (Bell et al.1995). This suggests that insoluble S is mobilized from mature leaves into other plant parts. However, it is not clear whether this is a temporal response or a response to S stress, since measurements of plants that were maintained at a constant level of S were not reported (Sunarpi and Anderson 1996).

On the other hand, the resulted were indicated that most of soybean lines observed clearly same band intensity for 7S and 11S globulins (Figure 2). From the results it was clear that, the accumulation of storage proteins in soybean seeds were controlled via sulfur assimilation during first stages of plant growth and therefore, some varieties without sulfur fertilizer showing tolerant to sulfur defect and were accumulated the 7S and 11S proteins compared with treated one (Figure 2). Responses to S fertilization in terms of storage protein concentration and quality differed among cultivars. Without sulfur applied, storage protein concentration in grains decreased slightly in all the cultivars, however grain quality remained stable as indicated by slight changes in 11S/7S ratio except for a remarkable reduction in Clark and Hokuiku10 cultivars (Table 6). For instance, in the

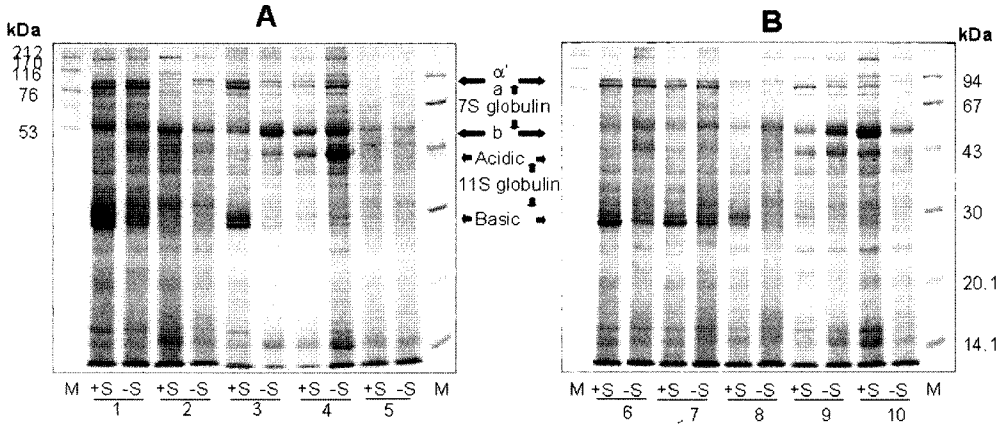

Figure 1. SDS-PAGE analysis of components of leaves storage proteins in soybean. Globulin fractions were isolated from soybean of sulfur fertilizer (+S) and without sulfur fertilizer (-S), and 25 mg of each fraction was fractionated by SDS-PAGE and then stained with CBB. (A). Plants from no. 1 to 5 and (B) from 6 to 10.

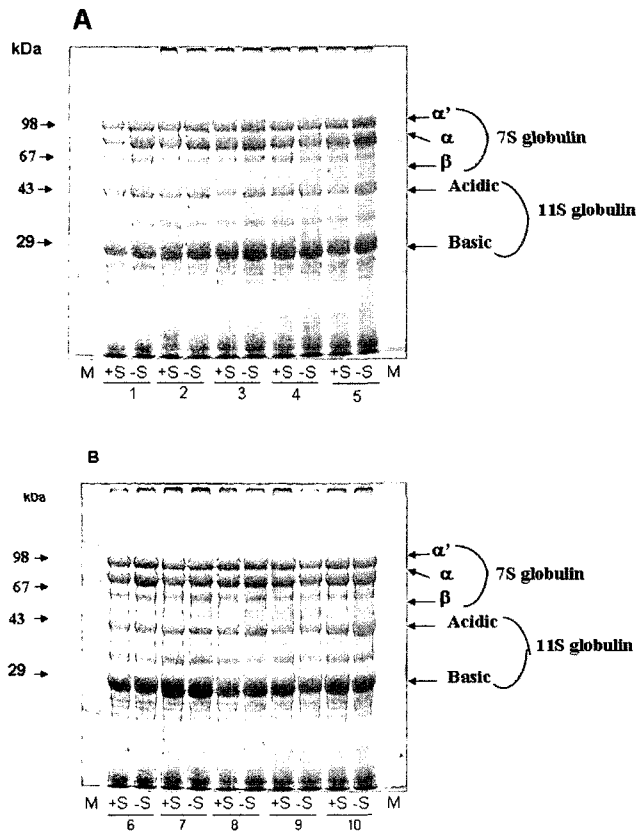

Figure 2. SDS-PAGE analysis of components of seed storage proteins in soybean. Globulin fractions were isolated from soybean of sulfur fertilizer (+S) and without sulfur fertilizer (-S), and 25 mg of each fraction was fractionated by SDS-PAGE and then stained with CBB. (A). Seeds plant from no. 1 to 5 and (B) from 6 to 10.

Tamahomare cultivar, storage protein concentration in seeds was higher but lower in quality than other ones in spite of sulfur conditions, on the other hand, in Fukuyutaka soybean, reverse trend was observed and Bragg cultivar demonstrated an intermediate level between these two varieties (Table 6).

These results suggest that response of soybean cultivars to sulfur nutrition differs genetically and sulfur nutritionally. El-Shemy et al. (2001) have found that regardless of large amount of N fertilizer applied under field conditions, pod removal treatment decreases its biomass production, however it leads to increase the grain protein concentration and also improves grain quality of the remaining pods. This finding strongly suggests that protein concentration and quality of grain are closely related to grain production.

Our results have shown that the sulfur partitioning among plant parts is also closely related to production and quality of grains: For instance, when more S was partitioned and maintained in leaves of Bragg soybean, the grain production increased but quality decreased (Table 6). On the other hand, when S partitioning to leaves was less but more in pods as observed in Fukuyutaka cultivar, grain production decreased due to termination of grain filling at earlier time as observed by leaf yellowing but quality was maintained higher even at sub-optimal S supply conditions.

Together these evidences, it can be speculated that the demand for S-containing amino acids by pods, major sink for proteins (Fujita et al.1989), rather than by source leaves may make such a difference. Presumably, it seems that the critical level for S-containing amino acids to express sink activity in pods is lower in Bragg than Fukuyutaka cultivars. The current results are consistent with previous findings that 11S protein composed of sulfur amino acid increases under an adequate supply of sulfur (Peak et al.1997; El-Shemy et al. 2001), while 7S protein with less sulfur amino acid maintained stable or rather increased at suboptimal supply of sulfur conditions (Table 6) (Peak et al. 1997). Thus, responses to sulfur nutrition in terms of 11S and 7S proteins are different among soybean cultivars, which results in differential response of grain quality.

The 7S and 11S globulin families are synthesized and deposited by a common intracellular mechanism that involves both posttranslational modifications and transport through organelles of the plant secretory pathway (Vitale et

Table 6. Effect of sulfur fertilizer on protein contents of soybean seeds

	Soybean of sulfur fertilizer (+S) and without sulfur fertilizer (-S) Percentage of seeds/dry weight																			
	1		2		3		4		5		6		7		8		9		10	
	-S	+S	-S	+S	-S	+S	-S	+S	-S	+S	-S	+S	-S	+S	-S	+S	-S	+S	-S	+S
Total proteins	40.2	41.6	39.8	40.4	38.9	42.2	38.5	43.6	39.4	42.8	38.1	43.2	39.3	41.7	38.2	42.8	39.1	43.6	38.4	42.7
Storage proteins	33.2	35.1	30.5	34.8	31.9	33.8	30.7	34.5	30.6	33.7	31.4	34.6	30.5	33.2	31.4	34.2	30.7	32.9	31.8	34.2
11S proteins	19.4	21.2	18.8	20.2	19.1	22.4	18.5	21.8	19.2	22.5	18.4	21.8	19.7	22.1	19.5	22.7	18.6	21.7	19.6	22.4
7S proteins	13.8	13.9	11.7	14.6	12.8	11.4	12.2	12.7	11.4	11.2	13.0	12.8	10.8	11.1	11.9	11.5	12.1	11.2	12.2	11.8
11S/7S ratio	1.40	1.52	1.60	1.38	1.49	1.96	1.52	1.72	1.68	2.0	1.4	1.70	1.82	1.99	1.64	1.97	1.54	1.94	1.60	1.90

al. 1993). The glycosylated 7S and non-glycosylated 11S globulins both undergo assembly into trimers in the lumen of the endoplasmic reticulum, a process seemingly required for their further intracellular transport into protein storage vacuoles of developing seeds (Chrispeels et al. 1982).

In plants that received adequate S during vegetative growth most of the S transported to grains was derived from soluble S; insoluble S (protein-S) was a minor contributor (Anderson and Fitzgerald 2001). The vegetative tissues of S-inadequate plants contained negligible amounts of soluble S, with the result that most of the S incorporated into the grains was derived from insoluble S (Anderson and Fitzgerald 2001).

It is clear that there are major differences in the responses of the cultivars to sulfur supplementation. In terms of total biomass, cultivar 6 (Bragg) increases its biomass by about 20% by adding sulfur while cultivar 1 (Tamahomare) increases its biomass by 23% with sulfur fertilization. The Bragg cultivar seems to be producing biomass at a near maximal rate in the absence of sulfur supplementation and addition of sulfur has little effect (less than 20% change) in total biomass, mature seed yield, storage protein, and the sulfur and nitrogen content of the seeds. For the three cultivars shown in Table 2, sulfur supplementation seems to bring total seed production close to similar values, betwee 31 and 38 grams dry weight per plant, while in the absence of sulfur the Fukuyutaka and Tamahomare cultivars have about half that yield in mature seeds (15 and 17 g/plant, respectively).

In terms of total protein and storage protein per gram dry weight of mature seeds, all ten cultivars are remarkably similar. There is less than a 10% increase in total seed protein with sulfur supplementation (Table 6). Sulfur supplementation does seem to increase the sulfur content of the seed protein with an average 16% increase for the 10 cultivars (Table 4).

From a functional viewpoint the plant sulphate transporter system has evolved to be an extremely efficient uptake system, with a high affinity for sulphate in the low micromolar range (Smith et al. 1997). This corresponds to typical soil solution sulphate concentrations and engineering for higher affinities may not be particularly useful even if technically feasible. Expression of the transporters is controlled by the nutritional status of the plant and the transporters are most highly expressed under S-limiting conditions also the complex regulation of sulfur amino acids synthesis (Hawkesford 2000; Riemenschneider et al. 2005).

The qualitative results of this investigation could have been anticipated, that sulfur supplementation would tend to increase the sulfur content of the plant, in particular in the storage proteins in the seed.

The data infer that under conditions that support uptake of S during generative growth the production of seeds with a high S content is important to improve the nutritional quality of soybean seeds. Through discussion based on the results in the present study, we can not work out the possibility that difference in source activity to assimilate sulfur-containing amino acids such as cysteine and methionine as well as the activities of roots to uptake and supply sulfur to source leaf may make differential response to S nutrition genetically.

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