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Effects of Nutritional Stress on the Storage Proteins of Soybeans[1]

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ABSTRACT

The effects of sulfur deficiency on the complement of proteins laid down in developing seeds of soybean (*Glycine max* L. Merr) have been examined. Sulfur deficiency caused a 40% decrease in the level of glycinins and a contrasting elevation in the level of β-conglycinins. The subunit composition of these proteins was also affected. There was in particular a 3-fold increase in the β-subunit of β-conglycinins in the sulfur-deficient seeds, and this accumulated largely as the B_0-isomer of β-conglycinins, a protein which while virtually devoid of methionine and cysteine retains the physical properties of a normal 7S storage protein. These data demonstrate that a high degree of selectivity can be exerted by environmental stress over the accumulation of proteins in developing seeds.

The amino acid compositions of glycinins and β-conglycinins, the major types of storage proteins of soybean (*Glycine max* L., Merr), vary sufficiently to affect their nutritional value in animal diets. Glycinins, in common with other legumin-like proteins from peas (*Pisum sativum*), lupin (*Lupinus angustifolius*), and broad bean (*Vicia faba*), contain more of the limiting amino acids cysteine and methionine, than the β-conglycinins and other vicilin-like proteins. The composition of the glycinins and β-conglycinins present in different soybeans is, however, not constant.

Both glycinins and β-conglycinins are families of proteins assembled from a number of different subunits and these subunits also differ in their contents of the sulfur containing amino acids. The range is from 0.6 to 3% (w/w) methionine plus cysteine between the extremes of glycinin subunits (12) and from virtually zero to 0.48% (w/w) for the subunits of the β-conglycinins (14). Such differences between subunits provide the basis for the production of storage protein complements of substantially different nutritional values, and this paper is part of a study on factors which can affect such production.

In other legume species, alteration in the growth conditions of plants from a normal nutrient supply to suboptimal conditions has been shown to alter the proportions of the major storage proteins present. In both lupin (3) and peas (7), sulfur deficiency specifically depressed the synthesis of particular sulfur-rich proteins. In peas, sulfur deficiency operated specifically by repression of the levels of legumin mRNA (1).

In this paper, we have investigated the ability of sulfur stress to alter the array of proteins present in soybeans, particularly the different isomers of β-conglycinin. We have shown that sulfur deficiency causes the production of greatly enhanced levels of the B_0-isomer[2] of β-conglycinin, a protein which, while virtually devoid of methionine and cysteine, retains the physical properties of a normal 7S storage protein (13, 16). We have shown in addition that the proteins in soybean are responsive to even the mild sulfur deficiencies likely to occur under field conditions, and predict that the already low cysteine and methionine contents of the soybean will be further depressed under these conditions to the detriment of its nutritional value.

MATERIALS AND METHODS

Plant Materials. *Glycine max* L. Merr cv Wayne was grown under artificial light 1000 μE m^{-2} s^{-1} on a 24°C/14 h d and 19°C/10 h night. Two nutrient regimes were imposed on the plants.

Specific Sulfur and Potassium Deficiency Trials. Plants were grown in acid-washed sand and pots flushed daily with one of three nutrient media and weekly with distilled H_2O. (a) Control plants received a complete nutrient solution containing ammonium nitrate 2 mM, calcium nitrate 4 mM, potassium chloride 5 mM, sodium hydrogen phosphate 0.375 mM, magnesium sulfate 0.375 mM, magnesium chloride 1.6 mM, sodium ferric EDTA 50 μM, boric acid 10 μM, manganous chloride 4.5 μM, zinc chloride 0.7 μM, cupric chloride 0.2 μM, and potassium iodide 0.2 μM. (b) Plants subjected to specific sulfur deficiency received an equivalent medium containing magnesium chloride in place of magnesium sulfate for the first 6 weeks of growth and then magnesium sulfate at a level of 0.094 mM to alleviate the severe symptoms of sulfur deficiency which appeared in the plants. (c) Plants subjected to specific potassium deficiency received an equivalent medium containing no potassium chloride.

Macronutrient Deprivation. Control plants were grown in a mixture of equal parts of red loam and sand supplemented with both solid nutrient mix (A) incorporated into the soil, and a further treatment every 2 weeks with 50 ml/plant of a nutrient solution (B) (Table I). Nutrient deficient plants were grown without the solid nutrient mix (A).

Harvesting. During flowering, pods were tagged for harvesting at intervals from 10 d after flowering to maturity. Seeds were removed from pods and weighed. Immature seeds were stored frozen. Mature pods were harvested from plants approximately 5 months after germination. Seeds were removed from the pods, dessicated for 2 d at room temperature, and stored frozen.

Protein Extraction. Following the removal of the seed coat and embryo, proteins were extracted from immature coytledons of different stages of development as previously described (2) in

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[2] Abbreviations: B_0-isomer and β_3-isomer refer to the same protein. The nomenclature of Yamauchi *et al.* (16), B_0-isomer has been used throughout this paper to describe the isomer of β-conglycinin which contains three identical β-subunits, and was independently named β_3-isomer (13).

Table I. *Inorganic Nutrient Supplements*

Solid Nutrient Mix (A)		Nutrient Solution (B)	
	g/kg soil mix		*mM*
Superphosphate	1	N	20
Lime	1	P	2.5
Dolomite	3	K	9
K_2SO_4	0.14	S	0.08
KNO_3	0.1		
$(NH_4)_2SO_4$	0.06		
$FeSO_4 \cdot 7H_2O$	0.1	Fe(EDTA)	0.02
$CuSO_4 \cdot 5H_2O$	0.07	Cu	0.02
$ZnSO_4 \cdot 7H_2O$	0.03	Zn	0.02
$MnSO_4 \cdot H_2O$	0.02	Mn	0.05
H_3BO_3	0.0004	B	0.005
$(NH_4)_2Mo\ O_4 \cdot 4H_2O$	0.001	Mo	0.0003

preparation for slab gel electrophoresis. Mature cotyledons were first finely ground, defatted with hexane, then homogenized in water and lyophilized to improve protein extraction. Protein was extracted from such meal in two ways.

For quantitative extraction, defatted meal (50 mg) was successively extracted four times by homogenization in a Dounce tissue homogenizer in 2.5 ml of 0.1 M Tris-HCl buffer (pH 8.0) containing 10% (w/v) NaCl, 0.02% (w/v) NaN_3, and 10 mM β-mercaptoethanol, and the slurry mixed for 2 h on an inversion mixer. Supernatants were collected after centrifugation in a Beckman microfuge.

For preparation of extracts suitable for subsequent fractionation of glycinin and the isomers of the β-conglycinins, proteins were initially extracted from 30-g samples of either control meal or sulfur-deficient meal in 30 mM Tris-HCl buffer (pH 8.0) containing 10 mM β-mercaptoethanol and 0.02% (w/v) NaN_3 as described previously (13).

Protein Fractionation. Glycinin and the B_0-isomer of β-conglycinin were first separated from other proteins in the total protein extracts by precipitation at pH 6.4 as previously described (13). The resultant precipitate (250 mg) was subjected to chromatography in 10 ml 35 mM K-phosphate buffer (pH 7.6) containing 0.4 M NaCl, 0.02% (w/v) NaN_3, and 10 mM β-mercaptoethanol on a 2.6 × 90-cm column of Sepharose 6B at 4°C.

Protein contents of all extracts were estimated by the method of Lowry *et al.* (5) after precipitation with 9% (w/v) TCA.

Electrophoretic Analysis. Protein extracts were heated at 80°C for 2.5 min in 30 mM Tris-HCl buffer (pH 8.0) containing 2% (w/v) SDS, 10% (w/v) glycerol, and 0.15 M β-mercaptoethanol to dissociate subunit complexes before electrophoresis on slab gradient (8–20%, w/v) polyacrylamide gels as described previously (10). Proteins were fixed and stained by the procedure of Gayler and Sykes (2) which permits the quantitative estimation of these storage proteins by densitometry.

Densitometry. Gels were scanned either on a LKB Lazer densitometer at 570 nM or on a Gelman DCD scanner at 575 nM and peak areas corrected in each case for differences in dye-binding capacity.

RESULTS

Effects of Sulfur Deprivation on Protein Composition at Maturity. When deprived of either sulfur or potassium, growth of the plants was greatly decreased and seed yields were reduced to less than half that of the controls. The composition of the storage proteins in the mature seeds of soybeans grown on media specifically lacking in either sulfur or potassium is shown in Figure 1 and Table II. Despite the drop in total seed yield, the level of protein per seed at maturity was almost unaffected by the different nutrient supplies. Likewise, the total content of the major storage proteins, that is, the sum of the glycinins and β-conglycinins

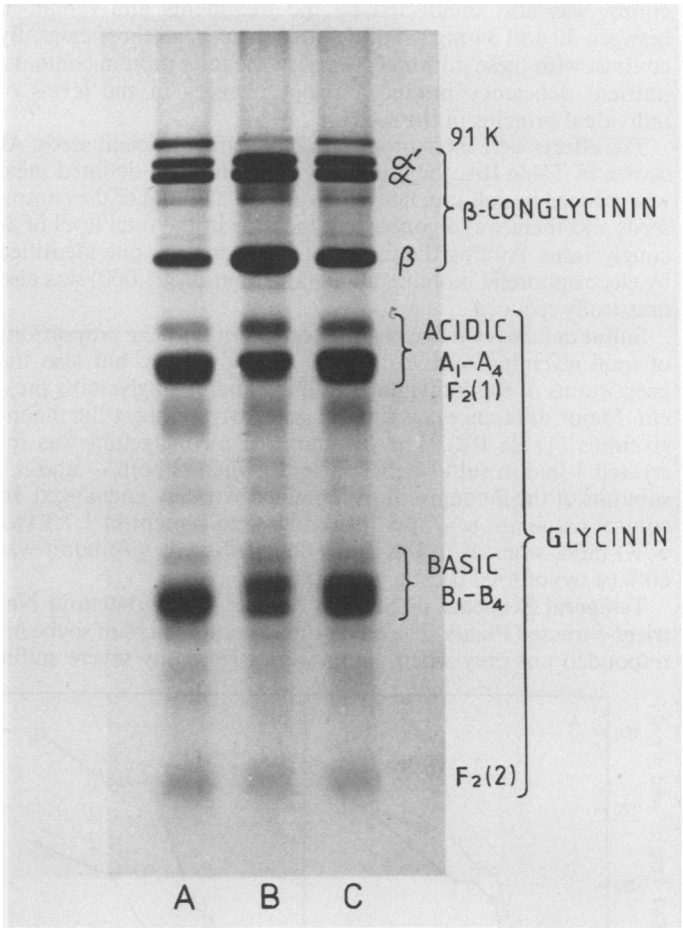

FIG. 1. Polypeptide complement of mature seeds from sulfur-deficient and potassium-deficient plants. Salt soluble proteins were extracted from mature seeds of (A) control, (B) sulfur-deficient, and (C) potassium-deficient plants and subjected (50 μg/track) to SDS-PAGE after reduction with β-mercaptoethanol.

Table II. *Protein Composition of Nutrient-Deficient Seeds*

Total salt soluble proteins were extracted from mature seeds from plants grown on acid-washed sand on completely defined media. Extracts were subjected to SDS-PAGE after reduction with β-mercaptoethanol as in Figure 1, and the proportions of particular proteins present were determined by densitometry of the stained gels.

	Protein Content		
	Control	−S	−K
	mg g^{-1} defatted meal		
A. Total extractable protein	44	38	46
Glycinins	18.3	10.3	20.4
β-Conglycinins	13.3	23.3	16.1
91,000 protein	2.0	0.1	0.8
Others	10.1	4.6	8.7
B. Dissociated subunits			
β-Conglycinins			
α'	4.4	5.5	3.1
α	4.3	3.9	6.1
β	4.6	13.9	6.9
Glycinins			
Acidic	12.2	7.1	13.8
Basic	6.0	3.2	6.9

cinins, was also unaffected by the treatments and remained between 32 and 34 mg g^{-1} of defatted meal in all three cases. By contrast with these minimal effects on the total protein contents, nutrient deficiency produced major changes in the levels of individual proteins in the seeds.

The effects were most pronounced in sulfur-deficient seeds. As shown in Table IIA, the total level of glycinins in defatted meal was reduced by sulfur deficiency to almost half that of the control seeds and there was a concurrent increase in the total level of β-conglycinins. Among the minor proteins present, one identified by electrophoretic mobility alone (apparent M_r 91,000) was also drastically reduced.

Sulfur deficiency in soybeans affected not only the proportions of total glycinins relative to total β-conglycinins, but also the proportions of the individual glycinins and β-conglycinins present. Major differences occurred particularly amongst the β-conglycinins (Table IIB). The β-subunit of β-conglycinin was increased 3-fold in sulfur-deficient seeds, whereas both α- and α′-subunits of the β-conglycinins remained virtually unchanged. In control tissue, α-, α′-, and β-subunits were present in 1:1:1 (w/w/w) ratio, whereas in the sulfur-deficient seeds, β-subunit was 60% (w/w) of total β-conglycinin.

Temporal Sequence of Storage Protein Accumulation in Nutrient-Stressed Plants. The seed protein complement of soybeans responded not only when plants were stressed by severe sulfur deficiency but also when plants were subjected to much milder conditions of nutrient deficiency. The effects on the composition of the storage proteins of the mild conditions of nutrient deficiency which developed when soybeans were grown in soil deprived only of a supplementary solid fertilizer mix are shown in Figures 2 and 3. In each case, the contents of the glycinins and β-conglycinins were determined throughout development. In control plants grown with full nutrient supply, glycinin and β-conglycinin were produced at similar rates throughout development. By maturity (70 d after flowering), the levels of the β-conglycinins and the glycinins in the seeds were almost identical (Fig. 2A). However, in plants grown under macronutrient deficiency, the rate of accumulation of the glycinins in the seeds was reduced, and that of the β-conglycinins substantially increased (Fig. 2B). Total protein content of the seeds, however, dropped only marginally from 36% (w/w) to 33% (w/w) of seed dry weight in the deficient plants.

The increase in the β-conglycinins was again almost entirely due to the excessive production of the β-subunit of the β-conglycinins, which increased from 38 mg g^{-1} dry weight in controls to 76 mg g^{-1} dry weight in nutrient-deficient plants. Such overproduction of β-subunit contrasts with the situation in plants receiving adequate nutrition where it has been previously shown that β-subunit production lags well behind the production of α- and α′-subunits throughout development and, even at maturity, reaches a level only the equivalent of the α- and α′-subunits (2, 6, 11). In plants deprived of macronutrients, the initial lag in β-subunit production was succeeded by a rapid increase in its rate of production and this was maintained at a high rate throughout development (Fig. 3).

Isomers of β-Conglycinin. Analysis under nondissociating conditions of the proteins present in mature seeds from normal and sulfur-deficient plants showed that the changes in subunit composition were also reflected in the isomers of β-conglycinin assembled from them. Of particular relevance was the B_0-isomer of β-conglycinin which had previously been shown to be a trimer composed entirely of β-subunits and to be a protein virtually free of cysteine and methionine. B_0-Isomer was separated from the other six β-conglycinins by isoelectric precipitation at pH 6.4 (13, 16). Because the other major storage proteins, the glycinins, also precipitated at pH 6.4, subsequent chromatography of this fraction on Sepharose 6B could be used to estimate the relative levels of the sulfur-rich glycinins and the sulfur-poor B_0-isomer. Samples prepared in this way from plants grown either on normal nutrient or on sulfur-deficient media are shown in Figure 4. The level of the B_0-isomer was in fact greatly increased relative to the glycinin content when sulfur was withheld from the plants,

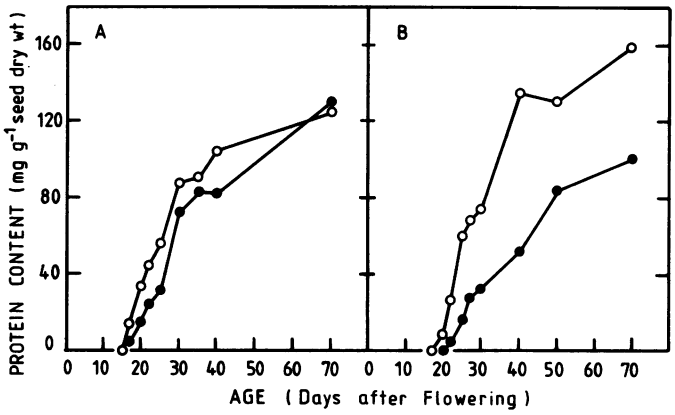

FIG. 2. β-Conglycinin and glycinin contents in developing seeds from normal and nutrient-deficient plants. The level of β-conglycinin (○) and glycinin (●) in seeds aged from 15 days after flowering to maturity (70 d after flowering) harvested from (A) control plants and (B) plants deprived of macronutrients as in "Materials and Methods" (2). Protein composition was determined as in Table II.

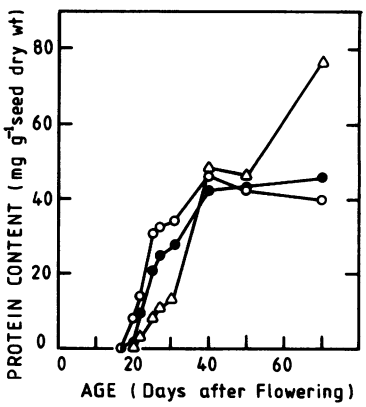

FIG. 3. Levels of the subunits of β-conglycinins in developing seeds from nutrient-deficient plants. The content of α- (○), α′- (●), and β- (△) subunits of β-conglycinin in seeds of plants deprived of macronutrients as in Figure 2.

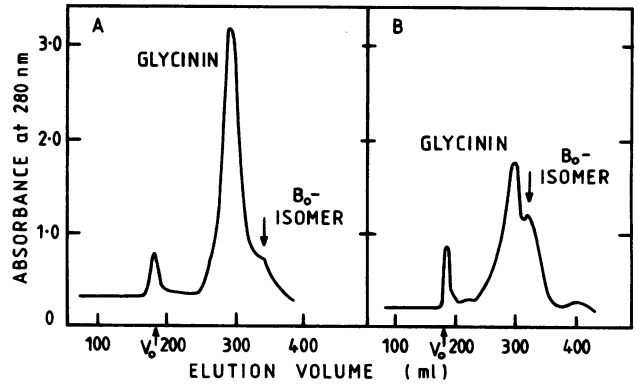

FIG. 4. Separation of B_0-isomer and glycinin by chromatography on Sepharose 6B. Protein precipitated at pH 6.4 from extracts of mature seeds from (A) control plants and (B) sulfur-deficient plants was chromatographed on Sepharose 6B (250 mg/column). (V_0), void volume. Peaks were identified by electrophoresis on SDS-PAGE.

indicating that most of the extra β-subunit produced in these plants was packaged as this pure trimer.

Further analysis of the B_0-isomer isolated from these sulfur-deficient seeds also confirmed that it contained the same amino and complement as normal β-subunit of β-conglycinin, and was again very low in methionine. The electrophoretic mobilities of the β-subunits from sulfur-deficient and normal plants were also identical. Therefore, even though it has been suggested that each of the subunits of the β-conglycinins of soybean may be encoded by small families of genes producing closely related subunit isotypes (9), the β-subunit which was produced in elevated levels under sulfur stress nevertheless appears to be the same protein as that produced normally.

DISCUSSION

The results of these trials show that a high degree of specificity is possible in the responses of storage proteins to environmental stress. The rates of accumulation of the individual polypeptide subunits from which the β-conglycinins are assembled responded individually to nutritional stress and therefore appeared to be under separate regulation. Since it has been shown (8) that each of the subunits of β-conglycinin is coded for by separate mRNAs, it would be possible for such specific regulation to occur in the same way as described for regulation of legumin in peas in response to equivalent sulfur deficiency. In peas, sulfur deficiency caused alteration in the level of legumin-specific mRNA (1). It is probable that both the modifications to the β-conglycinins and also the other alterations induced by sulfur deficiency, both within the glycinin family and of the protein of apparent M_r 91,000, all reflect similar alterations in specific mRNA levels.

Both the discrimination between the legumin-like and vicilin-like families of proteins and the discrimination between the different subunits within the β-conglycinin family appeared to be specific responses to lack of sulfur and presumably to lack of sulfur-containing amino acids in the plants. Consistent with this was the failure of potassium deficiency to produce equivalent changes (Fig. 1). In addition, the changes in the proportions of the two major types of storage proteins in sulfur-deficient soybeans closely followed the trends previously observed for equivalent legumin-like and vicilin-like proteins in other sulfur-deficient legumes (3, 7) and were by contrast the reciprocal of changes observed in soybean cultured in the presence of excess methionine (4). In all species examined so far, the legumin-like proteins which contain the higher levels of sulfur amino acids were suppressed in plants grown on low sulfur, while the vicilin-like proteins with their lower levels of sulfur amino acids were enhanced. The β-subunit of β-conglycinin, the only protein significantly elevated in sulfur-deficient soybean seed, is also the only storage protein in soybean which completely lacks methionine and cysteine. It is in addition the major protein whose production was specifically inhibited by the addition of excess methionine to soybean cotyledons in culture (4, 15). Repression and derepression of the synthesis of this particular protein in the presence and absence of methionine is therefore consistent with its amino acid composition.

The end result of the drastic alteration in available subunit types in nutrient-stressed soybean seeds was a change in the type of oligomeric protein packaged in the seed, and in particular the accumulation of high levels of the B_0-isomer of β-conglycinin. Despite being virtually totally deficient in methionine and cysteine B_0-isomer retains the physical properties of a normal vicilin-like protein. It retains the trimeric arrangement of subunits characteristic of the β-conglycinins, behaves on ultracentrifugation as a 7.5 S protein (13, 16) and presumably is capable of the same packing *in vivo* during dehydration as the rest of the β-conglycinin isomers. The capacity to form this type of protein when the production of the more sulfur-enriched proteins such as glycinin is inhibited by sulfur deficiency appears to ensure that nonsulfur amino acids can continue to be stored in the soybean seed as a protein with a normal packing structure even under severe sulfur stress, and this could be construed as an advantage for survival of the plant.

Finally, our results also suggest that since dramatic alterations in the subunit composition of the storage proteins occurred even when nutrient deprivation was so mild that no superficial signs of nutrient deficiency were visible on the plants, alteration in subunit composition and ultimately in nutritional value of the soybean may be more prevalent than previously suspected under prevailing conditions of crop management in the field.

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