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Stem Infusions Enhanced Methionine Content of Soybean Storage Protein[1]

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ABSTRACT

The quality of soybean (*Glycine max* [L.] Merrill) seed storage protein is limited by its low methionine (Met) content. Met supplementation of an *in vitro* soybean cotyledon culture has been shown to increase Met content by 21.9% due to an inhibition of the synthesis of the Met-devoid β subunit of 7S storage protein (JF Thompson *et al.* 1981, Phytochemistry 20: 941-945). The objective of this research was to determine if Met supplementation of intact plants would result in a similar improvement in soybean protein quality. A solution including 10 millimolar D,L malic acid plus 10 millimolar K_2HPO_4 with or without 20 millimolar D,L Met or 20 millimolar Na_2SO_4 was infused throughout seed development into lower stem internodes of soybeans (cv 'Williams 79' or 'Williams 82') grown under both greenhouse and field conditions. Pediatric intravenous kits were used to infuse an average of 51.2 milliliters per plant. Met content of whole soybean seeds from intact plants receiving Met infusions increased by as much as 22.7%. Even greater (up to 31.0%) increases in cysteine (Cys) content were noted, indicating that soybean plants are able to metabolize Met to Cys, or that supplemental Met allows Cys accumulation by some other mechanism. Electrophoretic patterns showed a dramatic decrease in the synthesis of the β subunit of 7S storage protein when Met was supplemented, and this effect was not confined to seeds at the lower nodes. In addition, seeds from upper compared to lower plant nodes (regardless of infusion treatment) had greater protein content (45.0 *versus* 41.6 w/w%), and different protein composition, as indicated by significantly different amino acid profiles. Methionine supplementation of intact soybean plants improved protein quality through an alteration in storage protein composition.

Although soybeans are a high protein grain crop, the quality of that protein is limited by its low Met[3] content, particularly when used in infant formulas (7) or in poultry rations (19, 21). Of the two major classes of soybean seed storage protein, 11S globulins contain more Met than do 7S globulins (10). In addition, the β subunit of 7S protein is devoid of Met (10). Two soybean cultivars with elevated 11S to 7S ratios have been found and were shown to contain 15% more Met than other cultivars (13). Thus, efforts to increase 11S to 7S ratios and/or to decrease β subunit content may be important in enhancing the nutritional quality of soybean seed protein.

The development by Thompson *et al.* (24) of an *in vitro* culture for soybean cotyledons has allowed direct Met supplementation. When 8.4 mM Met was added to the basal culture medium, Met content of protein increased 21.9% (25). A sharp decrease (11.4%) in Arg content implied that a shift in the proportions of the various storage proteins had occurred. SDS-PAGE of storage proteins indicated that the supplemental Met had inhibited β subunit synthesis (10). Further, supraoptimal levels of endogenous sulfate were not equivalent to Met supplementation (11). The inhibition of β subunit synthesis was due to the lack of functional mRNA, and not degradation of existing β subunit (3). The objective of this research was to determine if Met supplementation of intact plants would improve soybean protein quality. It has been shown that the appearance of β subunit in cotyledons of greenhouse-grown plants is closely related to a decrease in free Met (4). However, it is not known if additional Met would alter free Met content in the developing cotyledons. Intact plants could presumably process Met at various points after synthesis (or supplementation) and it may not be utilized directly by the developing cotyledons. For example, recent reports have shown that soybean seed coats metabolize allantoin and allantoic acid so extensively that only traces reach developing embryos (12, 22), even though these ureides comprise the major portion of N compounds transported in the xylem of N_2 fixing soybeans (17, 23). Other recent work has shown that amino acids exiting soybean leaf cells are incorporated into proteins for temporary storage in a specialized layer of leaf cells (29). In a preliminary study, foliar Met sprays did not alter soybean storage protein composition (26).

We report here the use of a novel stem infusion technique to introduce Met into intact soybean plants during seed development. Met content of seed storage protein was increased, no major increases in free Met in the seeds were noted, and β subunit synthesis was markedly depressed.

MATERIALS AND METHODS

Plant Culture. Soybeans (*Glycine max* [L.] Merrill cv 'Williams 79' [experiment 1] or 'Williams 82' [experiments 2-4]) were grown in four separate experiments in 1983 and 1984. Plants for experiments 1, 3, and 4 were grown in a temperature controlled (18-30°C) greenhouse under natural sunlight. Seeds were pregerminated on moist paper towels for 2 d at room temperature, inoculated with *Rhizobium japonicum* strain 3I1B 143, and planted (7 per pot) in 20.5 cm diameter plastic pots containing perlite in a modified Leonard jar system (28) using 6-L plastic outer containers. After emergence, all except two evenly matched plants were removed from each pot. The basal nutrient solution (27) included 1.70 mM sulfate, very close to the optimal sulfate level (1.75 mM) described earlier for soybean cotyledon culture (10). Plants for experiment 2 were field-grown at the Agronomy Research Center near Columbia on a Mexico silt loam soil (Udollic Ochraqualf). Phosphorus and K fertilizers were applied according to University of Missouri soil test recommendations at elemental rates of 95 and 146 kg ha^{-1}, respectively. Seeds were

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[3] Abbreviations: Met, methionine; Cys, cysteine.

not inoculated with rhizobia since soybeans had been grown on the site the previous year. Plots were planted at 30 seeds m^{-1} in four 7.62 m long rows spaced 76 cm apart. Twenty-four days after emergence, plants were thinned to 11 uniform plants m^{-1}. Thirty days after thinning, 12 bordered plants row^{-1} were marked for the infusion treatments, using a separate randomization in each replication. Weeds were controlled with a preemergence spray of 0.42 kg ha^{-1} linuron (3-[3-4-dichlorophenyl]-1-[methoxymethyl] acetanilide) and hand weeding as necessary. A wire supported by posts set just outside both ends of one of the two central rows of each plot was used to suspend the stem infusion equipment. A total of 12 cm of irrigation water was applied in five weekly intervals during a dry period from 7/23 to 8/20, which corresponded to growth stages full bloom to mid podfill.

Pediatric intravenous kits (Travenol Laboratories, Inc., Deerfield, IL) were filled with 50 ml of the appropriate treatment solution, suspended 1.5 m above the base of the plants, and equipped with 2.54 cm 21 gauge needles. Soybean plants were prepared for infusions by first inserting a 21 guage needle at a downward angle of 40 to 60° above horizontal midway on an internode, starting between nodes 3 and 4 (node 1 = cotyledonary node) and progressing upward as the plants grew. The initial needle was withdrawn along with a tissue plug, leaving a cavity for the insertion of the functioning needle. After the valve was opened, the infusion site was closely monitored for leakage. If any leakage was noted, the valve was closed, and the entire infusion preparation was repeated in the same or next higher internode. Upon completion of each infusion period of 3 to 7 d, valves were closed and needles withdrawn from the plants. Amount of solution infused was calculated by difference between initial and remaining volumes. If evidence of microbial growth in the remaining solution was detected, intravenous kits were flushed with 95% ethanol and triple rinsed with deionized tap water before a new infusion was started. Infusion treatments included noninfused controls, buffer only controls, and buffer plus 20 mM D,L Met for all experiments. In addition, a buffer plus 20 mM Na_2SO_4 control was added for experiment 4. The buffer consisted of 10 mM D,L malic acid plus 10 mM K_2HPO_4. The pH of all infusion solutions was between 4.10 and 4.20.

Stem infusion treatments were imposed at the beginning of seed development, and continued until physiological maturity. The lower stem internodes served as the infusion region. Internodes infused were 3 to 8, 6 to 10, 4 to 8, and 4 to 8, and average solution uptake (excluding noninfused controls) was 53.0, 69.3, 46.5, and 36.0 ml $plant^{-1}$, for experiments 1, 2, 3, and 4, respectively. Each time a new infusion period was started, needles were moved up the plant to the adjacent internode. Infusions conducted in this manner would be likely to result in distribution of metabolites throughout the soybean shoots for the following reasons: (a) soybean leaf vascular bundle traces pass downward through two stem internodes, then anastomize with the traces from other leaves (1), (b) xylem to phloem transfer has been identified in the legume *Lupinus albus* L. (18), and (c) xylem to phloem transfer was indicated to occur in soybeans by the model of Layzell and LaRue (16). Experiments 1 and 4 were replicated eight times and experiments 2 and 3 were replicated six times.

Amino Acid Analyses. Finely ground (40 mesh) seed from two plants per treatment from each experiment was subjected to cation exchange analysis of total (protein-bound plus free) amino acids using a Beckman B121M amino acid analyzer. Duplicate samples were oxidized in performic acid to provide valid Met and Cys values. For experiment 1, samples represented a uniform mixture of all seed produced by a given plant. For experiments 2 and 3, samples included only seed from the second and third nodes above the last infusion internode (nodes 12 and 13 for experiment 2, and nodes 10 and 11 for experiment 3). Two samples from each plant of experiment 4 were chosen to represent lower (nodes 5 and 6) and upper (nodes 12 and 13) plant nodes. In addition, duplicate samples from the buffer only and buffer plus 20 mM Met treated plants of experiment 1 were analyzed for free amino acids. Samples used in this analysis were extracted for 30 min in 0.1 N HCl, and an aliquot of each sample was treated with sulfosalicylic acid, and included norleucine as an internal standard. Following this extraction, free amino acids were analyzed using the same procedures discussed above for total amino acids.

Electrophoresis of Proteins. SDS-PAGE was a modification of the procedure of Laemmli (15), using an SE 600 Series slab gel electrophoresis unit (Hoefer Scientific Instruments, San Francisco, CA). Approximately 20 mg of ethyl ether defatted seed sample from experiment 1 plants were suspended in 10 ml of a sample buffer consisting of 0.0625 M Tris-HCl (pH 6.6), 2% (w/v) sodium lauryl sulfate, 10% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol, and 0.001% (w/v) bromophenol blue prepared from reagents obtained from Sigma. All other reagents were electrophoresis grade from Biorad. Proteins were dissociated by heating to 80°C for 15 min. The stacking gel was 6% (w/v) acrylamide in 0.5 M Tris-HCl (pH 6.8) and the separation gel had a gradient of 8 to 16% (w/v) acrylamide in 1.5 M Tris-HCl (pH 8.8). Protein standards (Sigma; Fig. 1 for details) were run concurrently. Gels were stained in 0.4% (w/v) Coomassie blue G-250 in 70% (v/v) $HClO_4$, and destained in 5.0% (v/v) glacial acetic acid.

RESULTS AND DISCUSSION

Met content increased sharply in whole soybean seeds from intact plants infused with solutions containing Met in all three greenhouse studies (experiments 1, 3, and 4), but no significant increase was noted in Met content of field-grown soybeans (experiment 2) (Tables I and II). Differences did not appear to be related to planting date. Increases in Met levels ranged from 22.7, 16.4, and 8.7% for greenhouse-grown soybeans in experiments 1, 3, and 4, respectively, to as low as 4.0% for the field-grown soybeans of experiment 2 (Table III). When a similar amount of sulfur was infused as sulfate rather than Met, Met levels were not affected (Table I), probably indicating that plant sulfate levels did not limit Met synthesis and incorporation into storage protein.

Holowach *et al.* (10) recently reported that Met supplementation of an *in vitro* cotyledon culture resulted in large (60-fold) increases in intracellular concentrations of free Met. Therefore, additional analyses were conducted to determine if Met infusions had influenced free Met content, since values in Tables I and II represented total (protein-bound plus free) amino acid levels. Duplicate seed samples from the same pairs of plants analyzed for total amino acids in the buffer and buffer plus 20 mM Met treatments of experiment 1 (shown in Table I) were analyzed for free amino acids. Met content of the free amino acid pool did not depend on treatment and averaged only 0.22 mol % compared to much higher average levels of 33.06, 14.02, 12.12, 7.97, and 7.31 mol % for Arg, Val, Asp, Thr, and Ser, respectively. Thus, Met supplementation of intact plants clearly did not alter free Met concentrations of the resulting seed.

The failure of Met infusions of field-grown soybeans to significantly increase Met content of resulting seed may be partially explained by higher yield levels (32.58 g $plant^{-1}$) recorded in experiment 2 compared to the lower yield levels (6.65, 13.66, and 9.02 g $plant^{-1}$, respectively) recorded for experiments 1, 3, and 4. When total infused Met was compared to total seed Met (both expressed in μmol Met $plant^{-1}$), the ratio of infused:seed Met was calculated to be 0.91 for the field-grown plants of experiment 2, but 2.62, 2.07, and 2.76 for the greenhouse-grown plants of experiments 1, 3, and 4, respectively. Thus, the failure of Met infusions to significantly improve Met content of field-grown soybeans may simply represent a dilution effect. Efforts

Table I. *Effect of Stem Infusion Treatments on Amino Acid Profiles of Seed from Greenhouse-Grown Soybeans (Experiments 1 and 4)*

Amino Acid	Stem Infusion Treatment								
	Experiment 1, 1983[a]				Experiment 4, 1984[a]				
	None	Buffer[b]	Buffer + 20 mM Met	LSD (0.10)	None	Buffer	Buffer + 20 mM Met	Buffer + 20 mM Na_2SO_4	LSD (0.10)
	mol %[c]								
Asp + Asn	11.51 ± 0.03[d]	11.51 ± 0.02	11.57 ± 0.01	NS	11.59 ± 0.02	11.54 ± 0.04	11.62 ± 0.01	11.58 ± 0.01	NS
Thr	4.25 ± 0.09	4.23 ± 0.04	4.34 ± 0.00	NS	4.28 ± 0.02	4.31 ± 0.04	4.30 ± 0.04	4.27 ± 0.03	NS
Ser	6.05 ± 0.10	6.16 ± 0.03	6.19 ± 0.15	NS	6.31 ± 0.04	6.30 ± 0.05	6.32 ± 0.04	6.39 ± 0.02	NS
Glu + Gln	17.16 ± 0.10	17.32 ± 0.04	17.03 ± 0.12	NS	17.08 ± 0.05	16.97 ± 0.12	17.05 ± 0.07	17.13 ± 0.09	NS
Pro	5.94 ± 0.09	5.83 ± 0.01	5.83 ± 0.09	NS	5.82 ± 0.04	5.88 ± 0.05	5.90 ± 0.05	5.85 ± 0.04	NS
Gly	7.59 ± 0.01	7.50 ± 0.03	7.61 ± 0.04	0.05	7.38 ± 0.03	7.45 ± 0.04	7.41 ± 0.03	7.42 ± 0.05	NS
Ala	6.26 ± 0.02	6.24 ± 0.03	6.36 ± 0.05	NS	6.35 ± 0.03	6.40 ± 0.07	6.35 ± 0.04	6.33 ± 0.03	NS
Cys	0.76 ± 0.02	0.69 ± 0.02	0.95 ± 0.01	0.02	0.83 ± 0.02	0.85 ± 0.02	0.96 ± 0.03	0.86 ± 0.03	NS
Val	5.67 ± 0.07	5.61 ± 0.02	5.57 ± 0.12	NS	5.63 ± 0.03	5.67 ± 0.06	5.58 ± 0.03	5.58 ± 0.02	NS
Met	1.17 ± 0.03	1.16 ± 0.02	1.43 ± 0.00	0.06	1.25 ± 0.02	1.27 ± 0.04	1.37 ± 0.03	1.26 ± 0.02	0.08
Ile	4.68 ± 0.01	4.65 ± 0.03	4.62 ± 0.07	NS	4.81 ± 0.03	4.79 ± 0.03	4.72 ± 0.03	4.75 ± 0.01	NS
Leu	7.84 ± 0.01	7.99 ± 0.00	7.79 ± 0.05	0.11	7.94 ± 0.02	7.89 ± 0.01	7.78 ± 0.02	7.88 ± 0.01	0.15
Tyr	2.77 ± 0.00	2.79 ± 0.00	2.77 ± 0.02	NS	2.81 ± 0.00	2.82 ± 0.02	2.77 ± 0.02	2.80 ± 0.01	NS
Phe	4.13 ± 0.03	4.19 ± 0.00	4.01 ± 0.02	0.07	4.14 ± 0.01	4.13 ± 0.01	4.05 ± 0.02	4.12 ± 0.02	0.09
His	2.41 ± 0.01	2.37 ± 0.00	2.39 ± 0.00	NS	2.32 ± 0.01	2.31 ± 0.01	2.30 ± 0.01	2.30 ± 0.01	NS
Lys	5.87 ± 0.01	5.78 ± 0.02	5.92 ± 0.07	NS	5.75 ± 0.00	5.79 ± 0.04	5.79 ± 0.03	5.77 ± 0.04	NS
Arg	5.95 ± 0.05	5.98 ± 0.04	5.62 ± 0.04	0.03	5.70 ± 0.05	5.62 ± 0.10	5.73 ± 0.09	5.69 ± 0.07	NS

[a] Experiments 1 and 4 are presented together since both were planted approximately July 1. [b] Buffer consisted of 10 mM D,L-malic acid plus 10 mM K_2HPO_4. [c] Calculation of mol % did not include tryptophan, which was not measured. [d] Each value represents the mean of determinations from two separate plants. Standard errors follow each mean.

Table II. *Effect of Stem Infusion Treatments on Amino Acid Profiles of Seed from Soybeans Grown in the Field or Greenhouse (Experiments 2 and 3)*

Amino Acid	Stem Infusion Treatment							
	Experiment 2, field[a]				Experiment 3, greenhouse[a]			
	None	Buffer[b]	Buffer + 20 mM Met	LSD (0.10)	None	Buffer	Buffer + 20 mM Met	LSD (0.10)
	mol %[c]							
Asp + Asn	11.47 ± 0.06[d]	11.48 ± 0.01	11.48 ± 0.01	NS	11.56 ± 0.07	11.47 ± 0.03	11.62 ± 0.01	NS
Thr	4.37 ± 0.03	4.35 ± 0.01	4.34 ± 0.02	NS	4.30 ± 0.02	4.36 ± 0.01	4.31 ± 0.01	NS
Ser	6.39 ± 0.11	6.36 ± 0.02	6.16 ± 0.08	NS	6.41 ± 0.04	6.33 ± 0.10	6.31 ± 0.03	NS
Glu + Gln	17.32 ± 0.05	17.20 ± 0.10	17.18 ± 0.15	NS	17.15 ± 0.05	16.85 ± 0.02	16.92 ± 0.02	0.16
Pro	5.80 ± 0.03	5.82 ± 0.05	5.81 ± 0.00	NS	5.71 ± 0.07	5.80 ± 0.07	5.80 ± 0.02	NS
Gly	7.54 ± 0.03	7.53 ± 0.02	7.51 ± 0.02	NS	7.54 ± 0.01	7.56 ± 0.01	7.52 ± 0.01	NS
Ala	6.50 ± 0.03	6.49 ± 0.02	6.48 ± 0.06	NS	6.50 ± 0.02	6.60 ± 0.05	6.50 ± 0.03	NS
Cys	0.86 ± 0.00	0.87 ± 0.01	0.93 ± 0.04	NS	0.77 ± 0.01	0.76 ± 0.00	0.99 ± 0.06	0.16
Val	5.54 ± 0.13	5.60 ± 0.01	5.70 ± 0.04	NS	5.66 ± 0.08	5.68 ± 0.09	5.57 ± 0.04	NS
Met	1.25 ± 0.01	1.25 ± 0.03	1.30 ± 0.06	NS	1.16 ± 0.00	1.16 ± 0.00	1.35 ± 0.03	0.07
Ile	4.72 ± 0.08	4.77 ± 0.01	4.85 ± 0.02	NS	4.85 ± 0.06	4.86 ± 0.08	4.84 ± 0.02	NS
Leu	7.74 ± 0.00	7.75 ± 0.00	7.74 ± 0.03	NS	7.88 ± 0.03	7.87 ± 0.01	7.73 ± 0.04	NS
Tyr	2.82 ± 0.02	2.82 ± 0.02	2.78 ± 0.03	NS	2.88 ± 0.03	2.91 ± 0.00	2.84 ± 0.00	NS
Phe	4.10 ± 0.01	4.08 ± 0.03	4.08 ± 0.02	NS	4.25 ± 0.06	4.24 ± 0.02	4.08 ± 0.03	NS
His	2.31 ± 0.01	2.30 ± 0.01	2.34 ± 0.02	0.02	2.36 ± 0.02	2.34 ± 0.01	2.35 ± 0.00	NS
Lys	5.71 ± 0.01	5.74 ± 0.02	5.81 ± 0.04	NS	5.45 ± 0.19	5.74 ± 0.01	5.79 ± 0.02	NS
Arg	5.56 ± 0.02	5.57 ± 0.01	5.50 ± 0.06	NS	5.58 ± 0.00	5.48 ± 0.01	5.47 ± 0.01	0.03

[a] Experiments 2 and 3 are presented together since both were planted approximately May 15. [b] Buffer consisted of 10 mM D,L-malic acid plus 10 mM K_2HPO_4. [c] Calculation of mol % did not include tryptophan, which was not measured. [d] Each value represents the mean of determinations from two separate plants. Standard errors follow each mean.

to infuse more Met by increasing concentrations up to 100 mM were unsuccessful, due to limited solution uptake.

Met levels of control plants for these experiments ranged from 1.16 to 1.27 mol %, roughly 20% higher than the control cotyledon culture of Thompson *et al.* (25). However, they used a different cultivar ('Provar'), and the fact that cultivars differ in Met content has long been established (14). Also, their cotyledon culture did not include the embryonic and seed coat proteins that our intact seeds did. While basal Met levels varied between our experiments and those of Thompson *et al.* (25), the maximal Met increase recorded in experiment I (22.7%) was comparable to their reported increase (21.9%).

Table III. *Relative Changes in Amino Acid Profiles of Soybean Cotyledon Cultures in Response to 8.4 mM Met in the Culture Media (data of Thompson et al. [25]) and in Intact Soybean Plants in Response to Stem Infusions of 20 mM Met (Experiments 1–4)*

Amino Acid	Cotyledon Culture (Ref. 25)	Whole Plant Culture			
		Exp. 1[a]	Exp. 3	Exp. 4	Exp. 2
		% change due to added Met[b]			
Asp + Asn	+3.0a[c]	+0.5	+0.9	+0.4	0.0
Thr	+4.2a	+2.4	−0.5	+0.3	−0.5
Ser	+3.5	+1.4	−0.9	−0.2	−3.4
Glu + Gln	−2.1b	−1.2	−0.5c	−0.1	−0.5
Pro	−0.3	−0.9	+0.8	+0.9	0.0
Gly	+4.6a	+0.9b	−0.4	−0.1	−0.3
Ala	+2.9b	+1.8	−0.8	−0.2	+2.3
Cys	ND[d]	+31.0a	+29.4c	+16.1	+7.5
Val	+0.6	−1.2	−1.8	−0.8	+2.3
Met	+21.9a	+22.7a	+16.4b	+8.7c	+4.0
Ile	0.0	−1.0	−0.3	−1.3	+2.2
Leu	−1.2	−1.6c	−1.8	−1.6c	−0.1
Tyr	0.0	−0.4	−1.9	−1.4	−1.4
Phe	−2.1a	−3.6b	−3.9	−1.9c	−0.2
His	−5.6b	0.0	0.0	−0.4	+1.5b
Lys	+2.0	+1.6	+3.5	+0.3	+1.5
Arg	−11.4a	−5.8a	−1.1b	+1.1	−1.2

[a] Experiments 1, 2, 3, and 4 (as described in "Materials and Methods") are ranked from left to right in descending order of relative shifts in Met content. [b] Relative changes expressed as percent change in mol percent of each amino acid in response to Met supplementation to cotyledon culture or to intact plants compared to control levels. For the data of Thompson *et al.* (25), the control amino acid profile was that of cotyledons cultured in the absence of Met. For our whole plant cultures, the control level for a given experiment was calculated as the mean of all treatments which did not include Met. Mol percent calculation differed slightly between cotyledon and whole plant culture in that cotyledon culture data did not include Cys, while whole plant data did. Neither group measured Trp. Moreover, the cotyledon culture included only cotyledonary proteins, while the whole plant culture included embryonic, seed coat, and cotyledonary proteins. [c] a, b, and c refer to significant differences from the control amino acid levels at probabilities of 1, 5, and 10%, respectively. [d] Not determined.

The levels of other amino acids were also influenced by Met infusions (Tables I–III). Levels of Cys were increased most, and exceeded the relative increases in Met for each experiment, reaching 31.0% in experiment 1 (Table III). Possibly, these soybean plants were able to convert some of the excess infused Met to Cys, or infused Met allowed Cys accumulation by a sparing effect. Previous research with other plant systems has indicated that plants are unable to synthesize Cys from Met. Giovanelli and Mudd (8) purified spinach β-cystathionase and found no γ-cystathionase activity, indicating no conversion of cystathionine to cysteine. Likewise, although *Lemna* plants are able to make this conversion of cystathionine to cysteine, the rates measured were too slow to support growth (5).

Our results are consistent with the report of Holowach *et al.* (11), which indicated that soybean cotyledons grew vigorously when Met was the only sulfur source included in the culture medium. Citing their own unpublished results, Holowach *et al.* (11) concluded that this reverse flow of S from Met to Cys only occurred under S limiting conditions, since ^{35}S label in Met added to cotyledon cultures with optimal sulfate levels was not incorporated into Cys residues of newly synthesized 7S storage protein β subunits. However, our intact plants may have metabolized Met to Cys, even when nutrient solution sulfate levels appeared to be optimal (1.7 mM), or Met may have allowed the accumulation of Cys by some other mechanism. This indicates further potential for improvement in soybean protein quality beyond that afforded by the elimination of β subunit synthesis, since Cys can have a sparing effect on the Met requirements of humans (6) and chicks (9).

Levels of other amino acids were influenced to a lesser degree than those of Met and Cys. Also, as the increases in these sulfur-containing amino acids declined, levels of fewer of the other amino acids were altered. For experiment 1, the other significant changes were decreases in Arg, Phe, and Leu, and a slight increase in Gly (Tables I and III). Nearly all of the significant changes due to added Met in experiment 1 were identical in direction and similar in magnitude to the changes due to added Met previously reported by Thompson *et al.* (25). For example, Arg fell by 5.8% in experiment 1 compared to an 11.4% decrease in their work. The strong implication that storage protein composition was altered in the system of Thompson *et al.* (25) has been verified by SDS-PAGE for both cotyledon culture (10) and Met-infused plants (see below). Experiments 2 to 4 resulted in smaller increases in Met and Cys, paralleled by fewer significant changes in other amino acids. This result was not unexpected, since shifts in other amino acids were apparently due to the supression of β subunit synthesis by Met or a Met-metabolite (11). As Met levels to which developing seeds were exposed declined, it follows that synthesis of β subunit would be less inhibited, and therefore result in smaller changes in the levels of other amino acids.

SDS-PAGE of whole seed proteins from experiment 1 revealed a dramatic inhibition of β subunit production in plants which received stem infusions including Met (Fig. 1). When electrophoresis lanes F and M (buffer plus Met treated plants) are contrasted against lanes B and I (noninfused controls) or lanes D and K (buffer only controls), it is clear that the β subunit virtually disappeared when Met was included in the infusion solution. When sample solution volume loaded on the gel was doubled

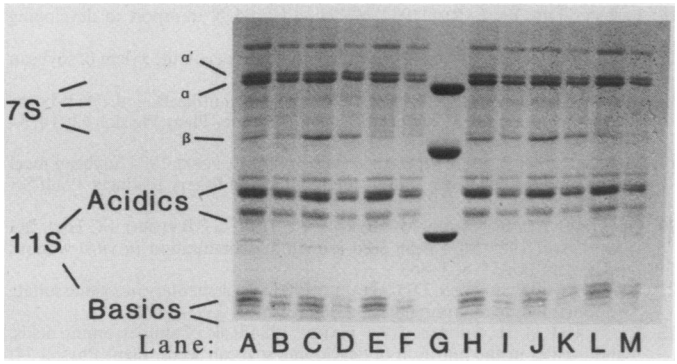

FIG. 1. SDS-PAGE patterns of 7S and 11S storage protein subunits taken from greenhouse-grown soybeans which received stem infusions of a buffer consisting of malate plus K_2HPO_4 with or without Met throughout seed development (experiment 1). Electrophoresis was according to Laemmli (15) as described in "Materials and Methods." Each sample included approximately 1 μg protein μl solution^{-1} loaded on to the gel. Protein group (7S or 11S) and subunits are indicated at the left. Lane assignments and loaded sample amounts were as follows: A and H, 50 μl from noninfused controls; B and I, 25 μl from noninfused controls; C and J, 50 μl from plants with buffer (10 mM D,L-malic acid plus 10 mM K_2HPO_4) only; D and K, 25 μl from buffer only plants; E and L, 50 μl from plants treated with buffer plus 20 mM D,L Met; F and M, 25 μl from buffer plus Met plants. Lanes A to F represent one replication of each of the three treatments, and lanes H to M represent a second replication of the same three treatments imposed on a separate set of three plants. Lane G was loaded with 50 μl of the following protein standards (Sigma) at the concentration of approximately 2 mg protein band^{-1}: top, bovine albumin (M_r = 66 kD); middle, egg albumin (M_r = 45 kD); bottom, carbonic anhydrase (from bovine erythrocytes, M_r = 29 kD). Proteins electrophoresed were extracted from seed of the same plants (experiment 1) for which amino acid profiles are reported in Table I.

from 25 to 50 μl, an identical pattern was detected (compare lanes E and L [buffer plus Met plants] with lanes A and H [noninfused controls] or lanes C and J [buffer only controls]).

Some doubt has existed that the results of Thompson *et al.* (25) with an *in vitro* culture could be duplicated *in vivo*. However, our results, using a whole plant system, confirm their findings indicating a dramatic inhibition of β subunit production in response to Met supplementation. Previous efforts to influence soybean storage protein composition of whole plants through foliar Met sprays (26) or through applications of high rates of S fertilizers to field-grown soybeans (2) had not succeeded. Our stem infusion technique, as well as the embryo culture of Obendorf *et al.* (20), which permits the growth of soybean seeds to maturity without pods, both provide methodologies to investigate the entire developmental course of storage protein composition through maturation, a feature lacking in cotyledon culture.

Met infusions into lower stem internodes might be expected to influence storage protein composition and Met content only in seed from pods attached to lower plant nodes, since Met concentrations would seem likely to be most elevated near the site of infusion. Therefore, to determine if the infused Met had been distributed preferentially to these lower nodes, seeds from nodes 5-6 (center of infused region) and nodes 12-13 (top two nodes) of experiment 4 plants were harvested separately and analyzed for amino acid content. Total protein content, when averaged across the four infusion treatments, was significantly ($P < 0.01$) higher in seed from upper compared to lower nodes (45.0 and 41.6 w/w %, respectively). In addition, mol % of 7 of the 17 amino acids measured varied with node sampled (Table IV). Seed from upper nodes had higher Arg and Glu + Gln content (3.8 and 1.0%, respectively), but lower content of Met,

Table IV. *Inherent Differences in the Amino Acid Profiles of Greenhouse-Grown Soybean Seeds from Lower and Upper Plant Nodes (Experiment 4)*

Amino Acid	Plant Portion[a]		
	Lower	Upper	LSD (0.10)
	mol %[b]		
Asp + Asn	11.57 ± 0.02[c]	11.60 ± 0.01	NS
Thr	4.33 ± 0.02	4.25 ± 0.02	0.04
Ser	6.33 ± 0.03	6.33 ± 0.03	NS
Glu + Gln	16.97 ± 0.06	17.14 ± 0.05	0.08
Pro	5.85 ± 0.04	5.88 ± 0.02	NS
Gly	7.46 ± 0.02	7.37 ± 0.02	0.03
Ala	6.41 ± 0.03	6.30 ± 0.02	0.04
Cys	0.89 ± 0.03	0.87 ± 0.02	NS
Val	5.63 ± 0.03	5.60 ± 0.02	NS
Met	1.32 ± 0.02	1.26 ± 0.02	0.05
Ile	4.76 ± 0.03	4.77 ± 0.01	NS
Leu	7.88 ± 0.03	7.87 ± 0.02	NS
Tyr	2.81 ± 0.01	2.79 ± 0.01	0.02
Phe	4.10 ± 0.02	4.11 ± 0.01	NS
His	2.31 ± 0.01	2.30 ± 0.01	NS
Lys	5.79 ± 0.02	5.75 ± 0.02	NS
Arg	5.58 ± 0.04	5.79 ± 0.04	0.05

[a] Lower plant nodes represented by seeds from nodes 5 and 6; upper plant nodes by seeds from top two nodes (12 and 13). [b] Calculation of mol % as described in Table I footnote. [c] Each value is the mean of eight observations. Standard errors follow each mean.

Thr, Ala, Gly, and Tyr (4.5, 1.8, 1.7, 1.2, and 0.7%, respectively). While samples from upper and lower nodes were not assessed for relative protein subunit levels, this comparison of amino acid profiles suggests that subunit composition differs with nodal position. Since seeds and leaves at upper nodes are developmentally younger than seeds and leaves at lower nodes of these indeterminate soybeans, it is not entirely unexpected that seed protein composition might differ.

While Met infusions increased overall Met content in experiment 4 (see Tables II and III), that increase was not confined to the lower nodes. When Met-infused plants were compared to all other infusion treatments, Met content of lower and upper nodes was increased by 4.7 and 9.1%, respectively (data not shown). Thus, supplemental Met may have been preferentially transported to upper nodes. None of the other infusion treatments in this experiment significantly changed the amino acid profiles of seed from nodal positions considered separately, even though some whole plant influences were detected (Table I).

The seed storage protein composition of intact soybean plants was successfully altered by direct Met supplementation through the use of a novel stem infusion technique. This indicates that the suppression of β subunit synthesis by Met (or a Met metabolite) previously reported in an *in vitro* system (10) is also operative *in vivo*. Infused Met was not confined to the nodal region infused, but was extensively transported to upper nodes. Finally, the stem infusion technique used in this research may have other applications. For example, small quantities of plant growth regulators or intermediates in biochemical pathways under investigation could be supplied to plants without the sometimes erratic uptake experienced with spraying, leaf abrasion, or soil applications.

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