JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY



# Seed Storage Protein Deficiency Improves Sulfur Amino Acid Content in Common Bean (*Phaseolus vulgaris* L.): Redirection of Sulfur from γ-Glutamyl-*S*-methyl-cysteine

MEGHAN TAYLOR,[†,‡] RALPH CHAPMAN,[†] RONALD BEYAERT,[§] CINTA HERNÁNDEZ-SEBASTIÀ,[†] AND FRÉDÉRIC MARSOLAIS[†,*]

Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, 1391 Sandford Street, London, Ontario, N5V 4T3, Canada, and Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre, Research Farm, Shafer Road, P.O. Box 186, Delhi, Ontario, N4B 2W9, Canada

The contents of sulfur amino acids in seeds of common bean (*Phaseolus vulgaris* L.) are suboptimal for nutrition. They accumulate large amounts of a γ-glutamyl dipeptide of *S*-methyl-cysteine, a nonprotein amino acid that cannot substitute for methionine or cysteine in the diet. Protein accumulation and amino acid composition were characterized in three genetically related lines integrating a progressive deficiency in major seed storage proteins, phaseolin, phytohemagglutinin, and arcelin. Nitrogen, carbon, and sulfur contents were comparable among the three lines. The contents of *S*-methyl-cysteine and γ-glutamyl-*S*-methyl-cysteine were progressively reduced in the mutants. Sulfur was shifted predominantly to the protein cysteine pool, while total methionine was only slightly elevated. Methionine and cystine contents (mg per g protein) were increased by up to ca. 40%, to levels slightly above FAO guidelines on amino acid requirements for human nutrition. These findings may be useful to improve the nutritional quality of common bean.

**KEYWORDS: Cysteine; γ-glutamyl-*S*-methyl-cysteine; *S*-methyl-cysteine; *Phaseolus vulgaris*; seed storage protein deficiency; sulfur amino acid content**

## INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the primary grain legume for human consumption worldwide and is an important source of vegetable protein, particularly in the developing world (*1*). The contents of sulfur amino acids in seeds of common bean and other legume crops are suboptimal for nutrition (*2*). Legume seeds often accumulate a variety of nonprotein amino acids, which may serve as nitrogen storage and chemical defense compounds. Nonprotein nitrogen, including free amino acids, constitutes a significant fraction of total nitrogen in seeds of common bean (ca. 8−14%) (*2*). *Phaseolus* and several *Vigna* species are characterized by the accumulation in seeds of high levels of the nonprotein amino acid, *S*-methyl-Cys, in the form of a γ-Glu dipeptide (*3*). Seeds of *Vicia narbonensis* contain a related, toxic dipeptide, γ-Glu-*S*-ethenyl-Cys (*4*). γ-Glu-*S*-methyl-Cys is stable under conditions resembling the stomach environment but is cleaved in the rat intestine, releasing free *S*-methyl-Cys (*5*). *S*-Methyl-Cys cannot substitute for Cys or Met in the diet, as it fails to elicit a growth response in animals. When fed with casein, *S*-methyl-Cys at doses of 0.3% and higher reduces food intake and weight gain in rats and is associated with enlargement of the kidney (*5*).

Different strategies have been used for the improvement of sulfur amino acid content in grain legumes. Transgenic approaches rely on the expression of sulfur-rich proteins and/or metabolic engineering of sulfur amino acids (*6–8*). The expression of foreign proteins is limited by sulfur supply and often results in a shift of sulfur from endogenous, sulfur-rich proteins (*9, 10*). In soybean, the improvement of Met content is an important objective for feed use. Transgenic expression of Brazil nut 2S albumin increased Met content by 26% (*11*), whereas expression of 15 kDa δ-zein increased Met and Cys contents by 20 and 35%, respectively (*12*). With 11 kDa δ-zein, the Met content was increased in the alcohol-soluble protein fraction but not in the seed (*13*). In common bean, the expression of Brazil nut 2S albumin increased Met content by 20% (*14*). Experiments in lupine and chickpea showed that expression of sunflower seed albumin can stimulate sulfur assimilation in seeds, resulting in a 90% increase in Met content, with a 10%

* To whom correspondence should be addressed. Tel: 519-457-1470. Fax: 519-457-3997. E-mail: marsolaisf@agr.gc.ca.

† Southern Crop Protection and Food Research Centre, London.

‡ Permanent address: Department of Biochemistry, Medical Sciences Building, University of Western Ontario, London, Ontario, N6A 5C1, Canada.

§ Southern Crop Protection and Food Research Centre, Research Farm, Delhi.

10.1021/jf800787y CCC: $40.75 Published 2008 by the American Chemical Society
Published on Web 06/28/2008

reduction in Cys content (*15–17*). In comparison, soybean and *V. narbonensis* seeds accumulate little sulfate, and homoglutathione (homoGSH) is the main form of sulfur imported by the developing soybean embryo (*18*). In *V. narbonensis*, coexpression of Brazil nut 2S albumin and a feedback-insensitive, bacterial Asp kinase increased Met and Cys content by 100 and 20%, respectively (*19*). Analysis of sulfur balance indicated that 70% of the increase was explained by an enhanced supply of organic sulfur. Decreases in γ-Glu-*S*-ethenyl-Cys (up to 2-fold) and free thiols, mainly γ-Glu-Cys and GSH, accounted for the remainder of the increase in sulfur amino acid content.

Traditional breeding methods have also been used to improve amino acid composition. Isolation of mutant lines of soybean resistant to ethionine enabled increases of seed Met and Cys contents by ca. 20% (*20*). Quantitative trait loci controlling sulfur amino acid content in soybean seeds were also recently identified (*21*). The maize *opaque-2* mutant provides a unique example where a change in the composition of endogenous proteins improves the levels of an essential amino acid, Lys, present at suboptimal levels in grain (*22*). The mutation affects a transcription factor required for the expression of 22 kDa α-zeins. The mutant has a similar protein content as wild-type. Compensation by nonzein proteins is associated with increased Lys content by 2-fold in the original mutant. The *opaque-2* mutant has inferior agronomic characteristics associated with its starchy endosperm phenotype, but integration of modifier loci can restore the endosperm texture, while keeping the high Lys content. This strategy has been used to breed nutritionally balanced "Quality Protein Maize", important in developing countries. In comparison, soybean mutants integrating deficiencies in the major seed storage proteins, glycinin and β-conglycinin, have normal levels of sulfur amino acids (*23*).

The objective of this study was to evaluate the impact of seed storage protein deficiency on protein accumulation and amino acid composition in mature seeds of common bean, with a particular emphasis on sulfur amino acids. An additional objective was to clarify a possible role of γ-Glu-*S*-methyl-Cys as a storage form of sulfur. Three genetically related lines integrating a progressive deficiency in major seed storage proteins were characterized. Osborn et al. previously described their generation (*24*). The SARC1 line contains the arcelin-1 variant introduced from a wild accession (G12882) into the navy bean cultivar Sanilac. SARC1 contains the S-type phaseolin derived from Sanilac and phytohemagglutinin and arcelin derived from the wild parent, due to tight linkage between phytohemagglutinin and arcelin genes (*25, 26*). Recessive null alleles conferring phaseolin or phytohemagglutinin deficiency, from *P. coccineus* and "Great Northern 1140", respectively, were introgressed in the SARC1 background. SMARC1-PN1 is deficient in phaseolin, while SMARC1N-PN1 is deficient in all three major seed storage proteins. The three lines share a similar level (ca. 85%) of the parental Sanilac background. To our knowledge, this is the first report of a significant improvement in sulfur amino acid content in seeds of common bean based on natural genetic variation in seed storage protein composition.

## MATERIALS AND METHODS

**Plant Material and Growth.** Seeds of common bean (*P. vulgaris* L.) lines SARC1, SMARC1-PN1, and SMARC1N-PN1 (white navy bean type) were provided by Dr. Thomas C. Osborn, University of Wisconsin (Madison, WI). Seeds of cv. Sanilac were provided by Dr. Soon Park, Agriculture and Agri-Food Canada, Greenhouse and Processing Crops Research Centre (Harrow, ON). Plants were grown in the field in narrow rows in Saint-Thomas, ON, in 2005.

**Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis.** Seed samples (1.2 g) were ground in liquid nitrogen. Ground tissue (40 mg) was homogenized in 1 mL of 0.5× sample buffer [4% SDS (w/v), 25 mM Tris-HCl, pH 8.8, and 2.5% glycerol (v/v)] and immediately boiled for 5 min at 100 °C. Extracts were centrifuged at 17530*g* for 15 min. Protein in the extracts was quantified using the Bio-Rad Protein Assay solution (Mississauga, ON) and bovine serum albumin as standard. Protein samples (50 μg) were submitted to 12% SDS-PAGE and visualized by Coomassie staining.

**Carbon, Nitrogen, and Sulfur Analyses.** Replicate seed samples (10 g) were ground to less than 1 mm in a cyclone sample mill (Udy Corp., Fort Collins, CO) and freeze-dried. The total carbon, nitrogen, and sulfur was determined in samples of 200 mg of ground seed tissue by dry combustion with a LECO CNS-2000 Elemental Analyzer (LECO Corp., St. Joseph, MI) as outlined in the instruction manual and application note 203-821-002. Briefly, the sample was combusted in a high-oxygen atmosphere that converted any elemental carbon in the sample to $CO_2$, any elemental sulfur in the sample to $SO_2$, and any elemental nitrogen in the sample into $N_2$ and $NO_x$. The $CO_2$ and $SO_2$ generated during combustion were passed through an infrared detector to determine total carbon and sulfur in the sample. The $NO_x$ generated was reduced to $N_2$, and the total $N_2$ gas generated during the combustion procedure was detected by thermal conductivity, after removing any $CO_2$ and water from the sample gas.

**Quantification of Total and Soluble Protein Contents.** Replicate seed samples (1.2 g) were ground in liquid nitrogen. Total protein was extracted according to Fichtner et al. (*27*). Ground tissue (80 mg) was homogenized in 1 mL of 50 mM HEPES, pH 7.4, and 0.1% Triton X-100 (v/v). The extract was centrifuged at 17530*g* for 3 min, and the insoluble protein in the pellet was extracted in 1 mL of 0.1 N NaOH, which was added to the original supernatant. The final extract was clarified by centrifugation at 17530*g* for 3 min. Soluble protein was extracted according to VandenBosch et al. (*28*). Briefly, ground tissue (75 mg) was homogenized in 1 mL of 50 mM Tris-HCl, pH 7.5, 10 mM DTT, 0.5 M sucrose, 5 mM *p*-aminobenzamidine, and 5% polyvinylpolypyrrolidine (w/v). Thawed extracts were subjected to two freeze−thaw cycles and centrifuged at 17000*g* for 15 min at 4 °C. The supernatants were used for quantification with the Bio-Rad Protein Assay.

**Amino Acid and γ-Glu Dipeptide Quantification.** For analysis of free amino acids and γ-Glu dipeptides (*29*), replicate samples were prepared as for the quantification of total and soluble protein contents. Ground tissue (100 mg) was homogenized in 800 μL of methanol:chloroform:water (12:5:3 v/v). The extract was centrifuged at 17530*g* for 10 min. To separate the phases, 213 μL of water and 53 μL of chloroform were added to the supernatant. After centrifugation at 17530*g* for 10 min, the upper, aqueous phase was transferred and dried in a centrifugal evaporator for ca. 2.5 h. γ-Glu dipeptides were quantified in comparison with synthetic standards (Bachem, King of Prussia, PA). Quantification of the γ-Glu-*S*-methyl-Cys standard was verified after acid hydrolysis.

For analysis of total amino acids, replicate samples prepared for elemental analysis were used, prior to freeze-drying. Ground sample (ca. 0.5 mg) was hydrolyzed in the presence of 6 N HCl and 1% phenol (w/v). Hydrolysis was performed using a Pico-Tag Workstation (Waters, Mississauga, ON) for 24 h at 110 °C according to the manufacturer's instructions. NorLeu was used as an internal standard. Cys was detected separately as cysteic acid, after performic acid oxidation. Amino acid analysis was performed on a Waters Pico-Tag System. The sample was treated with a redrying solution consisting of methanol:water:triethylamine (2:2:1 v/v), vortexed, and dried under vacuum for 15 min. The sample was then derivatized for 20 min at room temperature with a solution made up of methanol:water:triethylamine:phenylisothiocyanate (PITC) (7:1:1:1 v/v). The derivatizing solution was removed under vacuum for 15 min. The derivatized sample was again washed with the redrying solution, vortexed, and dried under vacuum for 15 min. The derivatized sample was dissolved in sample diluent (pH 7.40), and an aliquot was injected into the column, running on a modified Pico-Tag gradient of 25 min. The column temperature was 47.5 °C. The derivatized amino acids were detected at 254 nm. The Waters Pico-

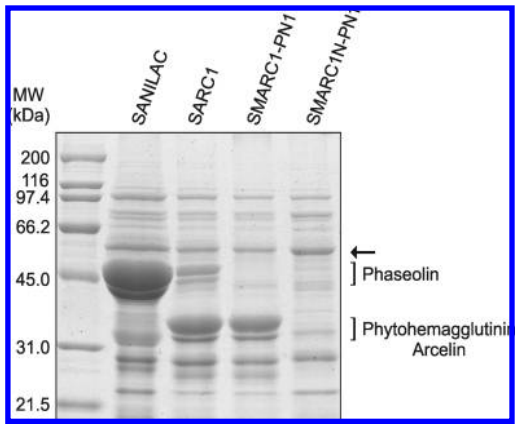


**Figure 1.** Protein profiles of seed storage protein-deficient lines and commercial parent Sanilac. The size of the markers is indicated on the left. The position of the major seed storage proteins is indicated by brackets. The position of a 54 kDa band up-regulated in F2 plants deficient in phaseolin, phytohemagglutinin, and arcelin (*35*) is shown by an arrow.

Tag system consisted of two model 510 HPLC pumps, Waters 2487 Dual Wavelength Absorbance Detector, Waters Sample Processor WISP 710B, Waters Column Heater, Waters Temperature Control Module, and an Agilent Hypersil ODS C18 column (4.6 mm × 250 mm) (Mississauga, ON). Data were collected, stored, and processed using Waters Millenium32 Chromatography software. Amino acid analyses were performed at the Advanced Protein Technology Centre, Hospital for Sick Children (Toronto, ON).

**Analysis of γ-Glu Dipeptides by Mass Spectrometry.** Fractions containing the two peaks of interest from the free amino acid analysis were examined by liquid chromatography−mass spectrometry. The system consisted of an Alliance 2690 HPLC/autoinjector and a model LCT orthogonal time-of-flight mass spectrometer (Waters). Chromatography was performed on a 2 mm × 150 mm, 5 μm Prodogy ODS(3) column (Phenomenex, Torrence, CA) fitted with a Security Guard cartridge using a gradient with solvent flowing at 0.2 mL $min^{-1}$. Solvent A was 10 mM ammonium acetate:acetonitrile (90:10, v/v), and solvent B was 10 mM ammonium acetate:acetonitrile (10:90, v/v). The gradient consisted of 100% A for 5 min, followed by a linear increase to 30% B at 15 min, and a second linear increase to 60% B at 25 min. A 1% solution (v/v) of ammonia was added to the column effluent at 20 μL $min^{-1}$, and total flow was passed through a standard nebulizer-assisted electrospray probe operated in negative ion mode, with nitrogen as the desolvation gas at 250 °C flowing at 455 L $h^{-1}$ and a potential of 2.7 kV applied to the capillary. The cone voltage was switched between 20 and 50 V at ca. 1 s intervals to permit observation of unfragmented and in-source collision-induced dissociation-produced fragment ions in the same chromatography run over the range 85−1500 *m/z*.

**Statistical Analysis.** One-way analysis of variance (ANOVA) was performed using the SuperANOVA statistical program (Abacus Concepts, Berkeley, CA).

## RESULTS

**Protein Profiles of Seed Storage Protein Deficient Lines.** Hartweck and Osborn had previously established the absence of phaseolin, phytohemagglutinin, and arcelin in SMARC1-PN1 and SMARC1N-PN1 by immunological methods (*30*). Genotypes were confirmed by SDS-PAGE of total protein extracts from mature seed. As seen in **Figure 1**, SARC1 contained much less phaseolin than the commercial parent Sanilac, in agreement with the observation that introgression of the arcelin-1 allele can lower phaseolin content from ca. 50 to 14% of total protein (*31*). Two major bands of 49 and 46 kDa were detected in SARC1, corresponding to γ- and α-β glycopolypeptides of S-type phaseolin, respectively (*32*). As expected, these bands were absent from the phaseolin-deficient lines, SMARC1-PN1 and SMARC1N-PN1. Phytohemagglutinin monomers are normally detected as two bands of 34 and 32 kDa (*33*) and arcelin-1 as a band of 36 kDa (*34*). Two prominent bands of 35 and 32 kDa corresponded to these proteins in SARC1 and SMARC1-PN1 but were absent in SMARC1N-PN1. A protein band of 54 kDa was previously shown to be up-regulated in F2 seeds combining the same alleles conferring deficiencies in phaseolin, phytohemagglutinin, and arcelin (*35*).

**Table 1.** Carbon, Nitrogen, and Sulfur Contents of Mature Seeds of Common Bean Lines[a]

| line | C (%) | N (%) | S (%) |
|---|---|---|---|
| SARC1 | 45.56 | 4.19 | 0.227 |
| SMARC1-PN1 | 45.61 | 4.64 | 0.236 |
| SMARC1N-PN1 | 45.18 | 4.28 | 0.232 |
| LSD | 0.11 | 0.15 | 0.007 |
| ANOVA *P* value | 0.0001 | 0.0002 | 0.046 |

[a] Values are expressed in % dry weight. Fisher's protected least significant difference (LSD) is expressed at $P \leq 0.05$; $n = 4$. Values for each *n* sample were the averages of duplicate measurements.

**Table 2.** Extractible Protein Contents of Mature Seeds of Common Bean Lines[a]

| line | total protein content (%) | soluble protein content (%) |
|---|---|---|
| SARC1 | 15.5 | **4.64** |
| SMARC1-PN1 | 14.8 | **5.82** |
| SMARC1N-PN1 | 17.8 | **7.40** |
| LSD | 2.4 | **0.87** |
| ANOVA *p* value | NS[b] | **0.0007** |

[a] Values are expressed in % of seed weight. NS, not significant; $n = 3$. Significantly different values are highlighted in bold. [b] Not significant after rank transformation of the data. Transformed mean values were as follows: SARC1, 4.3; SMARC1-PN1, 3.0; and SMARC1N-PN1, 7.7; LSD = 4.1.

**Nitrogen, Carbon, and Sulfur Contents and Protein Accumulation.** The three lines were previously shown to have similar nitrogen content in mature seed (*30*). The results of elemental analyses revealed minor differences among the three lines. Total nitrogen and sulfur contents were slightly elevated in the intermediate line SMARC1-PN1, by 10 and 4% as compared with wild-type SARC1, respectively (**Table 1**). The total carbon content was slightly lower in SMARC1N-PN1, by 1% as compared with SARC1. The total extractible protein content was not significantly different among the three lines (**Table 2**), but storage protein deficiency was associated with a progressive increase in soluble protein, up to 1.6-fold in the extreme line SMARC1N-PN1, as compared with SARC1.

**Free Amino Acids.** An analysis was performed to quantify individual free amino acids in mature seeds. Total free amino acids were significantly elevated in response to seed storage protein deficiency, by 2-fold in SMARC1N-PN1 as compared with wild-type SARC1 (mol/w) (**Table 3**). This is consistent with the 50% increase in total free amino acids, expressed as a percent of total nitrogen (w/w), reported for F2 seeds integrating the same mutations (*35*). All nitrogen-rich amino acids except Gln were elevated, particularly Arg (3.8-fold in SMARC1N-PN1 and 52% of total free amino acids) and Asn (1.6-fold) but also His and Lys. Central intermediates of nitrogen flow in amino acid biosynthesis, Ala, Asp, and Gly were also increased, as well as the Arg precursor, citrulline. The contents of all other free amino acids remained unchanged, with the notable exception of *S*-methyl-Cys, which decreased by 1.3-fold.

**γ-Glu Dipeptides.** The same procedure enabled the quantification of two abundant γ-Glu dipeptides present in seeds of

**Table 3.** Profiles of Free Amino Acids and γ-Glu Dipeptides in Mature Seeds of Common Bean Lines[a]

| line | Asp | Glu | Asn | Ser | Gln | Gly | His | citrulline |
|---|---|---|---|---|---|---|---|---|
| SARC1 | **0.32** | 0.66 | **1.2** | 0.075 | 0.067 | **0.12** | **0.46** | **0.048** |
| SMARC1-PN1 | **0.33** | 0.76 | **1.7** | 0.087 | 0.060 | **0.19** | **0.80** | **0.078** |
| SMARC1N-PN1 | **0.44** | 0.82 | **1.9** | 0.105 | 0.058 | **0.20** | **0.72** | **0.063** |
| LSD | **0.06** | 0.18 | **0.6** | 0.025 | 0.029 | **0.05** | **0.16** | **0.014** |
| ANOVA *P* value | **0.005** | NS | **0.03**[b] | NS | NS | **0.02** | **0.004** | **0.008** |

| line | Arg | GABA[c] | Thr | Ala | Pro | Tyr | *S*-methyl-Cys | Val |
|---|---|---|---|---|---|---|---|---|
| SARC1 | **1.9** | 0.16 | 0.14 | **0.31** | 0.39 | 0.084 | **0.097** | 0.24 |
| SMARC1-PN1 | **4.2** | 0.18 | 0.16 | **0.40** | 0.48 | 0.102 | **0.095** | 0.27 |
| SMARC1N-PN1 | **7.3** | 0.15 | 0.19 | **0.45** | 0.43 | 0.112 | **0.072** | 0.26 |
| LSD | **0.6** | 0.05 | 0.04 | **0.09** | 0.08 | 0.033 | **0.019** | 0.07 |
| ANOVA *P* value | **0.0001** | NS | NS | **0.02** | NS | NS | **0.04** | NS |

| line | Met | Ile | Leu | Phe | ornithine | Lys | total free amino acids |
|---|---|---|---|---|---|---|---|
| SARC1 | 0.011 | 0.070 | 0.086 | 0.21 | 0.021 | **0.027** | **6.8** |
| SMARC1-PN1 | 0.014 | 0.092 | 0.115 | 0.17 | 0.025 | **0.032** | **10.4** |
| SMARC1N-PN1 | 0.013 | 0.079 | 0.120 | 0.27 | 0.033 | **0.055** | **13.9** |
| LSD | 0.006 | 0.022 | 0.030 | 0.08 | 0.010 | **0.018** | **1.8** |
| ANOVA *P* value | NS | NS | NS | NS | NS | **0.02** | **0.0002** |

| line | γ-Glu-Leu | γ-Glu-*S*-methyl-Cys |
|---|---|---|
| SARC1 | **1.2** | **7.4** |
| SMARC1-PN1 | **2.0** | **6.0** |
| SMARC1N-PN1 | **2.2** | **4.0** |
| LSD | **0.17** | **1.2** |
| ANOVA *P* value | **0.0001** | **0.001** |

[a] Values are expressed in nmol $mg^{-1}$ seed weight; $n = 3$. [b] Significant after rank transformation of the data. Transformed mean values were as follows: SARC1, 2.5; SMARC1-PN1, 4.8; and SMARC1N-PN1, 7.6; LSD = 3.6. [c] γ-Aminobutyric acid.

common bean. Two prominent peaks detected by HPLC were confirmed as PITC derivatives of γ-Glu-*S*-methyl-Cys (retention time, 10.4 min) and γ-Glu-Leu (retention time, 15.4 min) by negative ion collision-induced dissociation mass spectrometry (**Figure 2**). Unfragmented ions at *m*/*z* of 398.5 and 394.6 had values expected for the $[M - H]^-$ of PITC-derivatized γ-Glu-*S*-methyl-Cys (398.4) and γ-Glu-Leu (394.3), respectively. Upon collision-induced dissociation, dipeptide derivatives appeared to decompose by first releasing the PITC group (neutral loss of 135) producing major ions for the $[M - H]^-$ of the underivatized dipeptide. Both dipeptides produced a major ion at *m*/*z* 128, presumably deprotonated pyroGlu, diagnostic of γ-Glu dipeptides (*36*). In the case of γ-Glu-Leu, a major ion at *m*/*z* 130 was observed, corresponding to the $[M - H]^-$ of Leu (**Figure 2b**). γ-Glu-Leu appeared to decompose readily by the loss of water and $CO_2$. In the case of γ-Glu-*S*-methyl-Cys, methanethiol also appeared to be released (neutral loss of 48) (**Figure 2a**).

Like free *S*-methyl-Cys, γ-Glu-*S*-methyl-Cys was progressively decreased with storage protein deficiency, up to 1.8-fold in SMARC1N-PN1 as compared with SARC1 (**Table 3**). The sulfur dipeptide was highly abundant, its content being approximately equal to that of total free amino acids in SARC1. Like for most variable free amino acids, γ-Glu-Leu content was increased in response to seed storage protein deficiency, by 1.8-fold in SMARC1N-PN1.

**Total Amino Acids.** These results prompted an analysis of total amino acids released after acid hydrolysis of mature seed tissue, to investigate whether sulfur accumulated as γ-Glu-*S*-methyl-Cys was transferred to the protein Cys or Met pools in the storage protein-deficient lines. The levels of total amino acids were similar between the three lines (**Table 4**), consistent with results obtained for total extractible protein contents (**Table 2**). A progressive increase in Arg (up to 1.6-fold) was accompanied by smaller decreases in several abundant amino acids, Ser, Val, Ile, Leu, and Phe in SMARC1N-PN1 (**Table 4**). Lys was significantly elevated only in the intermediate line SMARC1-PN1 (1.7-fold). The total Cys content was progressively increased, up to 1.7-fold, in response to storage protein deficiency. In comparison, Met was only slightly elevated in the mutants, by 1.2-fold in SMARC1-PN1 and 1.1-fold in SMARC1N-PN1. The levels of total *S*-methyl-Cys were reduced by 1.8-fold. Unexpectedly, its levels were ca. 3-fold higher than those of γ-Glu-*S*-methyl-Cys (**Tables 3** and **4**).

## DISCUSSION

The goal of this study was to evaluate the impact of seed storage protein deficiency on protein accumulation and amino acid composition in common bean. Three genetically related lines integrating a progressive deficiency in phaseolin, phytohemagglutinin, and arcelin were analyzed. Only minor differences were detected in total nitrogen, sulfur, and carbon contents in mature seed (**Table 1**). These may be related to differences in genetic background. The three lines had a similar total extractible protein content (**Table 2**) and total amino acid content (**Table 4**). These results confirmed that the absence of major seed storage proteins has little impact on seed protein content and is compensated by increases in other proteins (**Figure 1**) (*30*). Seed storage protein deficiency was associated with a progressive increase in soluble protein content (**Table 2**), suggesting an alteration in the pathways of protein deposition. Transgenic soybean deficient in the α- and α′-subunits of β-conglycinin accumulate proglycinin and store glycinin in ER-

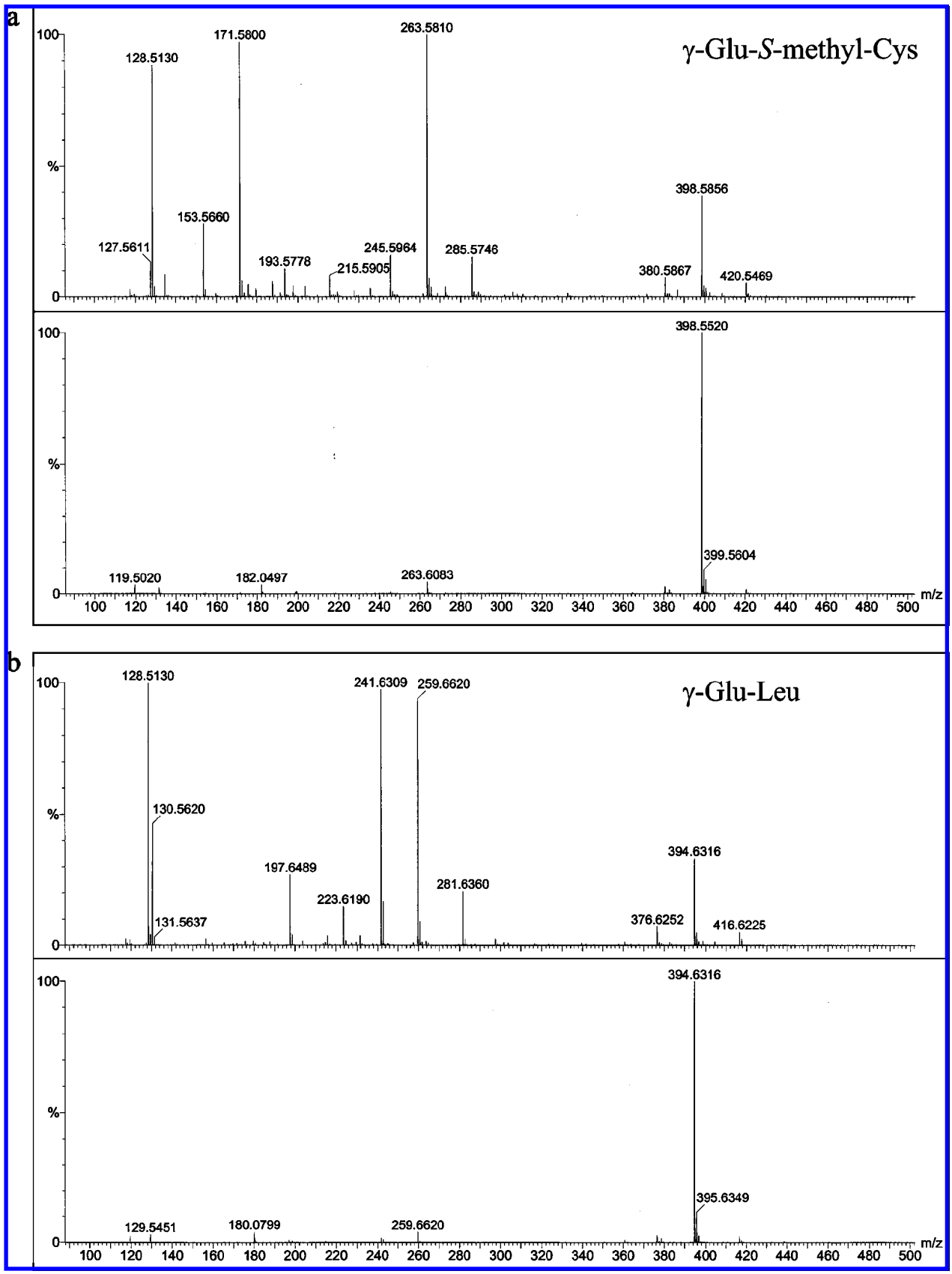


**Figure 2.** Negative ion mode mass spectra of PITC-derivatized γ-Glu-*S*-methyl-Cys (**a**) and γ-Glu-Leu (**b**). The top panel shows the spectrum obtained under conditions of collision-induced dissociation at a cone voltage of 50 V. The bottom panel shows the spectrum of the unfragmented ion at a cone voltage of 20 V.

derived protein bodies similar to those present in cereals, whereas seed storage proteins in soybean are normally deposited in Golgi-derived protein storage vacuoles (*37*). It is possible that phaseolin and lectin deficiency leads to similar changes in common bean, given the close genetic relatedness between the two species.

**Table 4.** Total Amino Acid Profiles in Mature Seeds of Common Bean Lines[a]

| line | Cys[b] | Asx | Glx | Ser | Gly | His | Arg | Thr | Ala | Pro |
|---|---|---|---|---|---|---|---|---|---|---|
| SARC1 | **20.8** | 252 | 228 | **155** | 126 | 46.4 | **110** | 98 | 131 | 79.7 |
| SMARC1-PN1 | **25.0** | 242 | 201 | **157** | 133 | 51.6 | **139** | 110 | 133 | 80.8 |
| SMARC1N-PN1 | **35.0** | 219 | 242 | **125** | 132 | 50.0 | **171** | 89 | 126 | 91.8 |
| LSD | **6.4** | 42 | 46 | **23** | 23 | 8.1 | **24** | 16 | 19 | 14.1 |
| ANOVA *p* value | **0.002** | NS | NS | **0.02** | NS | NS | **0.001** | NS[c] | NS | NS |

| line | Tyr | Val | Met | Ile | Leu | Phe | Lys | total protein amino acids | *S*-methyl-Cys |
|---|---|---|---|---|---|---|---|---|---|
| SARC1 | 45.0 | **122** | **15.1** | **80.8** | **134** | **71.7** | **65** | 1780 | **21.8** |
| SMARC1-PN1 | 47.1 | **130** | **18.3** | **93.6** | **136** | **95.2** | **108** | 1868 | **18.4** |
| SMARC1N-PN1 | 44.1 | **111** | **17.0** | **68.8** | **121** | **55.0** | **76** | 1772 | **12.7** |
| LSD | 5.9 | **12** | **1.7** | **4.4** | **8** | **7.6** | **18** | 237 | **2.5** |
| ANOVA *p* value | NS | **0.02** | **0.007** | **0.0001** | **0.0004** | **0.0001** | **0.001** | NS | **0.0001** |

[a] Values are expressed in nmol $mg^{-1}$ seed weight; $n = 4$. [b] All means were significantly different after rank transformation of the data; ANOVA $p \leq 0.0002$. Transformed mean values were as follows: SARC1, 2.5; SMARC1-PN1, 6.8; and SMARC1N-PN1, 10.2; LSD = 2.5. [c] Not significant after rank transformation of the data. Transformed mean values were as follows: SARC1, 5.8; SMARC1-PN1, 9.6; and SMARC1N-PN1, 4.1; LSD = 4.7.

Seed storage protein deficiency was partially compensated by an increase in several free amino acids and the dipeptide γ-Glu-Leu (**Table 3**). The pattern of changes in free amino acids was overall similar to that observed in soybean deficient in glycinin and β-conglycinin (*23*), namely, a large increase in Arg and a significant contribution from Asn. In *opaque-2* maize recombinant inbred lines deficient in 22 kDa α-zein, there is a large increase in Asp-derived free amino acids, including Lys, and in amino acids derived from glycolytic intermediates (Ser, Leu, Ala and Val) (*38*). Free Arg is also increased but not dominant, contrarily to soybean or common bean. For total amino acids, the large increase observed in Arg content (**Table 4**) is also similar to soybean, where it was hypothesized to function in maintaining seed nitrogen content (*23*). This increase in Arg was paralleled by a decrease of several abundant amino acids derived from organic acids, namely, Ser, Val, Ile, Leu, and Phe, whereas Glx is decreased in soybean (*23*). A large increase in Lys was observed only in the intermediate line SMARC1-PN1, probably determined in part by the amino acid composition of the proteins compensating for the lack of phaseolin in this line.

The most important finding from this study was the modulation of sulfur amino acids in response to seed storage protein deficiency. Sulfur from *S*-methyl-Cys and γ-Glu-*S*-methyl-Cys was shifted preferentially to the protein Cys pool in the storage protein deficient lines, with only a slight increase in the protein Met pool (**Tables 3** and **4**). Values measured for total *S*-methyl-Cys content in seeds of common bean range from 14 to 35 nmol $mg^{-1}$ (*39, 40*), as compared with 21.8 nmol $mg^{-1}$ for wild-type in this study (**Table 4**). Giada et al. (*3*) estimated the contents of γ-Glu-*S*-methyl-Cys and free *S*-methyl-Cys in different cultivars after acid hydrolysis of purified extracts. Mean values of 8.7 and 2.2 nmol $mg^{-1}$ were measured for γ-Glu-*S*-methyl-Cys and free *S*-methyl-Cys, respectively. As compared with this study (**Table 3**), the higher value for the free *S*-methyl-Cys content may have resulted from slow hydrolysis of the dipeptide in dilute acetic acid during purification. The value of 7.4 nmol $mg^{-1}$ measured for γ-Glu-*S*-methyl-Cys content in this study is consistent with these results. Using a slightly different extraction procedure (*41*), similar amounts of γ-Glu-*S*-methyl-Cys were detected in mature seeds of the BAT93 line ($9.4 \pm 0.8$ nmol $mg^{-1}$, average ± SD, $n = 3$) (Agnieszka Pajak and Frédéric Marsolais, unpublished results). Beside γ-Glu-*S*-methyl-Cys, other metabolites may contain *S*-methyl-Cys. Small amounts of γ-Glu-γ-Glu-*S*-methyl-Cys and *S*-methylhomoGSH have been detected in seeds of *Vigna radiata* (*41*). However, on the basis of the 3-fold higher content of total *S*-methyl-Cys as compared with γ-Glu-*S*-methyl-Cys (**Tables 3** and **4**), part of the *S*-methyl-Cys is present in other, possibly bound forms. For example, crystallographic and mass spectrometric studies have revealed that a lectin from *Canavalia gladiata* is purified from seeds in complex with the nonprotein amino acid, α-aminobutyric acid (*42*). In SMARC1N-PN1, the decrease in total *S*-methyl-Cys accounted for only ca. 60% of the increase in protein Cys, on a molar basis (**Table 4**). Decreases in γ-Glu-Cys and homoGSH may account, at least in part, for the remainder of the sulfur balance in SMARC1N-PN1.

The findings on increased Cys and Met content presented here might be useful to improve the nutritional quality of common bean. Cys is a semiessential amino acid and has a "sparing" effect on the amount of Met provided in the diet, decreasing the need for Met to Cys conversion via the reverse *trans*-sulfuration pathway (*43*). On the basis of a protein content of 25% (N × 6.25), the Met and cystine content was raised from 18.9 mg $g^{-1}$ protein in SARC1 to 26.8 mg $g^{-1}$ protein in SMARC1N-PN1 (ca. 40%), slightly above FAO guidelines of 25 mg $g^{-1}$ protein for human nutrition (*44*). Sulfur amino acid deficiency is prevalent in protein-energy malnutrition (*45*). For example, in populations subsisting on cassava, sulfur amino acid deficiency is associated with an inability to detoxify cyanide, leading to chronic neurotoxicity (*46*). Further experiments will be required to evaluate whether the high content of sulfur amino acids in SMARC1N-PN1 is associated with enhanced bioavailability and improved protein quality. Proof-of-concept studies to develop common bean cultivars with improved sulfur amino acid content would also require introduction of the phaseolin and phytohemagglutinin null alleles into a cultivated variety not having the arcelin-1 protein. To further improve sulfur amino acid content, at the expense of *S*-methyl-Cys (**Table 4**), seed storage protein deficiency could be combined with transgenic expression of a foreign, sulfur-rich protein.

The increased Cys content in SMARC1N-PN1 must be determined in part by the nature of the proteins synthesized in place of phaseolin and lectins and the metabolic plasticity enabling the shift of sulfur from γ-Glu-*S*-methyl-Cys to the protein Cys pool. Not surprisingly, both phaseolin and phytohemagglutinin are totally devoid of Cys. In a survey of a wide range of cultivated and wild *Phaseolus* and *Vigna* species, Baldi and Salamini (*39*) noted that Met content was relatively stable, whereas cystine and *S*-methyl-Cys contents were highly variable, with several *Vigna* species having only trace amounts of *S*-methyl-Cys. They proposed using interspecific hybrids for

improvement of sulfur amino acid content; however, strong incompatibility barriers limit this approach.

The results presented here provide insight into a possible biosynthetic pathway of γ-Glu-*S*-methyl-Cys. The preferential interconversion of sulfur stored as γ-Glu-*S*-methyl-Cys to the protein Cys pool suggests that *S*-methyl-Cys may arise from *S*-methylation of Cys during seed maturation. To date, a similar mechanism has been clarified only for *Se*-Cys. An *Se*-Cys *Se*-methyltransferase was purified and characterized from the legume *Astragalus bisulcatus*, a selenium hyperaccumulator (*47*). The enzyme had no catalytic activity toward Cys in vitro. However, transgenic expression in *Arabidopsis* was reported to lead to the production of equal amounts of *Se*-methyl-Cys and *S*-methyl-Cys (*48*). The corresponding recombinant *Se*-Cys *Se*-methyltransferase from broccoli was catalytically active with Cys but had a 40-fold higher substrate preference for *Se*-Cys (*49*). A related enzyme may be involved in the formation of γ-Glu-*S*-methyl-Cys during seed maturation in common bean. Following Cys *S*-methylation, the dipeptide may be formed by γ-Glu-Cys synthetase (*50*) or by transfer of the γ-Glu residue of homoGSH, catalyzed by γ-Glu transpeptidase (*51*). Beside Cys, γ-Glu-Cys or homoGSH constitute other possible methyl acceptors. An alternative pathway of *S*-methyl-Cys biosynthesis involves the condensation of methanethiol and *O*-acetylserine catalyzed *O*-acetylserine sulfhydrylase. Cleavage of Met by Met γ-lyase leads to the formation of *S*-methyl-Cys, possibly through the release of methanethiol (*52*). This reaction appears important under conditions of sulfate starvation in *Arabidopsis* (*53*). This route is less likely as there is no obvious source of methanethiol in developing seeds. The genetically related lines contrasted in γ-Glu-*S*-methyl-Cys content identified in this study will constitute a useful tool for biochemical and functional genomic studies of γ-Glu-*S*-methyl-Cys biosynthesis.

In summary, the present results show that combined deficiencies in phaseolin, phytohemagglutinin, and arcelin in seeds of common bean lead to a significant increase in sulfur amino acid content, particularly Cys, mostly at the expense of *S*-methyl-Cys and γ-Glu-*S*-methyl-Cys. These findings may provide a strategy to improve the nutritional quality of common bean, based on natural genetic variation in seed storage protein composition.

## ACKNOWLEDGMENT

We thank Luanne Bruneau and Dale MacArthur for technical assistance, Alex Molnar for preparation of figures, and Soon Park and Terry Rupert for plant growth.

## LITERATURE CITED

(1) Broughton, W. J.; Hernandez, G.; Blair, M.; Beebe, S.; Gepts, P.; Vanderleyden, J. Beans (*Phaseolus* spp.)—Model food legumes. *Plant Soil* **2003**, *252*, 55–128.

(2) Sathe, S. K. Dry bean protein functionality. *Crit. Rev. Biotechnol.* **2002**, *22*, 175–223.

(3) Giada, M. D. R.; Teresa, M.; Miranda, M.; Marquez, U. M. L. Sulphur gamma-glutamyl peptides in mature seeds of common beans (*Phaseolus vulgaris* L.). *Food Chem.* **1998**, *61*, 177–184.

(4) Enneking, D.; Delaere, I. M.; Tate, M. E. gamma-Glutamyl-*S*-ethenylcysteine: A dipeptide from *Vicia narbonensis*. *Phytochemistry* **1998**, *48*, 643–645.

(5) Padovese, R.; Kina, S. M.; Barros, R. M. C.; Borelli, P.; Marquez, U. M. L. Biological importance of gamma-glutamyl-*S*-methylcysteine of kidney bean (*Phaseolus vulgaris* L.). *Food Chem.* **2001**, *73*, 291–297.

(6) Galili, G.; Amir, R.; Hoefgen, R.; Hesse, H. Improving the levels of essential amino acids and sulfur metabolites in plants. *Biol. Chem.* **2005**, *386*, 817–31.

(7) Krishnan, H. B. Engineering soybean for enhanced sulfur amino acid content. *Crop Sci.* **2005**, *45*, 454–461.

(8) Beauregard, M.; Hefford, M. A. Enhancement of essential amino acid contents in crops by genetic engineering and protein design. *Plant Biotechnol. J.* **2006**, *4*, 561–574.

(9) Streit, L. G.; Beach, L. R.; Register, J. C.; Jung, R.; Fehr, W. R. Association of the Brazil nut protein gene and Kunitz trypsin inhibitor alleles with soybean protease inhibitor activity and agronomic traits. *Crop Sci.* **2001**, *41*, 1757–1760.

(10) Tabe, L. M.; Droux, M. Limits to sulfur accumulation in transgenic lupin seeds expressing a foreign sulfur-rich protein. *Plant Physiol.* **2002**, *128*, 1137–48.

(11) Townsend, J. A.; Thomas, L. A. Factors which influence the Agrobacterium-mediated transformation of soybean. *J Cell. Biochem. Suppl.* **1994**, *18A*, 78.

(12) Dinkins, R. D.; Reddy, M. S. S.; Meurer, C. A.; Yan, B.; Trick, H.; Thibaud-Nissen, F.; Finer, J. J.; Parrott, W. A.; Collins, G. B. Increased sulfur amino acids in soybean plants overexpressing the maize 15 kDa zein protein. *In Vitro Cell. Dev. Biol.-Plant* **2001**, *37*, 742–747.

(13) Kim, W. S.; Krishnan, H. B. Expression of an 11 kDa methionine-rich delta-zein in transgenic soybean results in the formation of two types of novel protein bodies in transitional cells situated between the vascular tissue and storage parenchyma cells. *Plant Biotechnol. J.* **2004**, *2*, 199–210.

(14) Aragao, F. J. L.; Barros, L. M. G.; de Sousa, M. V.; de Sa, M. F. G.; Almeida, E. R. P.; Gander, E. S.; Rech, E. L. Expression of a methionine-rich storage albumin from the Brazil nut (*Bertholletia excelsa* HBK, Lecythidaceae) in transgenic bean plants (*Phaseolus vulgaris* L., Fabaceae). *Genet. Mol. Biol.* **1999**, *22*, 445–449.

(15) Molvig, L.; Tabe, L. M.; Eggum, B. O.; Moore, A. E.; Craig, S.; Spencer, D.; Higgins, T. J. Enhanced methionine levels and increased nutritive value of seeds of transgenic lupins (*Lupinus angustifolius* L.) expressing a sunflower seed albumin gene. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 8393–8398.

(16) Tabe, L. M.; Droux, M. Sulfur assimilation in developing lupin cotyledons could contribute significantly to the accumulation of organic sulfur reserves in the seed. *Plant Physiol.* **2001**, *126*, 176–187.

(17) Chiaiese, P.; Ohkama-Ohtsu, N.; Molvig, L.; Godfree, R.; Dove, H.; Hocart, C.; Fujiwara, T.; Higgins, T. J.; Tabe, L. M. Sulphur and nitrogen nutrition influence the response of chickpea seeds to an added, transgenic sink for organic sulphur. *J. Exp. Bot.* **2004**, *55*, 1889–1901.

(18) Anderson, J. W.; Fitzgerald, M. A. Physiological and metabolic origin of sulphur for the synthesis of seed storage proteins. *J. Plant Physiol.* **2001**, *158*, 447–456.

(19) Demidov, D.; Horstmann, C.; Meixner, M.; Pickardt, T.; Saalbach, I.; Galili, G.; Muentz, K. Additive effects of the feed-back insensitive bacterial aspartate kinase and the Brazil nut 2S albumin on the methionine content of transgenic narbon bean (*Vicia narbonensis* L.). *Mol. Breed.* **2003**, *11*, 187–201.

(20) Imsande, J. Selection of soybean mutants with increased concentrations of seed methionine and cysteine. *Crop Sci.* **2001**, *41*, 510–515.

(21) Panthee, D. R.; Pantalone, V. R.; Sams, C. E.; Saxton, A. M.; West, D. R.; Orf, J. H.; Killam, A. S. Quantitative trait loci controlling sulfur containing amino acids, methionine and cysteine, in soybean seeds. *Theor. Appl. Genet.* **2006**, *112*, 546–553.

(22) Gibbon, B. C.; Larkins, B. A. Molecular genetic approaches to developing quality protein maize. *Trends Genet.* **2005**, *21*, 227–233.

(23) Takahashi, M.; Uematsu, Y.; Kashiwaba, K.; Yagasaki, K.; Hajika, M.; Matsunaga, R.; Komatsu, K.; Ishimoto, M. Accumulation of high levels of free amino acids in soybean seeds through integration of mutations conferring seed protein deficiency. *Planta* **2003**, *217*, 577–586.

(24) Osborn, T. C.; Hartweck, L. M.; Harmsen, R. H.; Vogelzang, R. D.; Kmiecik, K. A.; Bliss, F. A. Registration of *Phaseolus vulgaris* genetic stocks with altered seed protein compositions. *Crop Sci.* **2003**, *43*, 1570–1571.
(25) Osborn, T. C.; Blake, T.; Gepts, P.; Bliss, F. A. Bean arcelin 2. Genetic variation inheritance and linkage relationships of a novel seed protein of *Phaseolus vulgaris*. *Theor. Appl. Genet.* **1986**, *71*, 847–855.
(26) Kami, J.; Poncet, V.; Geffroy, V.; Gepts, P. Development of four phylogenetically-arrayed BAC libraries and sequence of the APA locus in *Phaseolus vulgaris*. *Theor. Appl. Genet.* **2006**, *112*, 987–998.
(27) Fichtner, K.; Quick, W. P.; Schulze, E. D.; Mooney, H. A.; Rodermel, S. R.; Bogorad, L.; Stitt, M. Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with "antisense" rbcS. v. relationship between photosynthetic rate, storage strategy, biomass allocation and vegetative plant growth at three different nitrogen supplies. *Planta* **1993**, *190*, 1–9.
(28) VandenBosch, K. A.; Bradley, D. J.; Knox, J. P.; Perotto, S.; Butcher, G. W.; Brewin, N. J. Common components of the infection thread matrix and the intercellular space identified by immunocytochemical analysis of pea nodules and uninfected roots. *EMBO J.* **1989**, *8*, 335–342.
(29) Hernández-Sebastià, C.; Marsolais, F.; Saravitz, C.; Israel, D.; Dewey, R. E.; Huber, S. C. Free amino acid profiles suggest a possible role for asparagine in the control of storage-product accumulation in developing seeds of low- and high-protein soybean lines. *J. Exp. Bot.* **2005**, *56*, 1951–1963.
(30) Hartweck, L. M.; Osborn, T. C. Altering protein composition by genetically removing phaseolin from common bean seeds containing arcelin or phytohemagglutinin. *Theor. Appl. Genet.* **1997**, *95*, 1012–1017.
(31) Romero, A. J.; Yandell, B. S.; Bliss, F. A. Bean arcelin 1. Inheritance of a novel seed protein of *Phaseolus-vulgaris* and its effect on seed composition. *Theor. Appl. Genet.* **1986**, *72*, 123–128.
(32) Brown, J. W. S.; Osborn, T. C.; Bliss, F. A.; Hall, T. C. Genetic variation in the subunits of globulin 2 and albumin seed proteins of French bean *Phaseolus-vulgaris*. *Theor. Appl. Genet.* **1981**, *60*, 245–250.
(33) Vitale, A.; Bollini, R. Legume storage proteins. In *Seed Development and Germination*; Kigel, J., Galili, G. Eds.; Marcel Dekker: New York, 1995; pp 73−102.
(34) Hartweck, L. M.; Vogelzang, R. D.; Osborn, T. C. Characterization and comparison of arcelin seed protein variants from common bean. *Plant Physiol.* **1991**, *97*, 204–211.
(35) Burow, M. D.; Ludden, P. W.; Bliss, F. A. Suppression of phaseolin and lectin in seeds of common bean *Phaseolus vulgaris* L.: Increased accumulation of 54 kDa polypeptides is not associated with higher seed methionine concentrations. *Mol. Gen. Genet.* **1993**, *241*, 431–439.
(36) Harrison, A. G. Characterization of alpha- and gamma-glutamyl dipeptides by negative ion collision-induced dissociation. *J. Mass Spectrom.* **2004**, *39*, 136–144.
(37) Kinney, A. J.; Jung, R.; Herman, E. M. Cosuppression of the alpha subunits of beta-conglycinin in transgenic soybean seeds induces the formation of endoplasmic reticulum-derived protein bodies. *Plant Cell* **2001**, *13*, 1165–1178.
(38) Wang, X.; Larkins, B. A. Genetic analysis of amino acid accumulation in *opaque-2* maize endosperm. *Plant Physiol.* **2001**, *125*, 1766–1777.
(39) Baldi, G.; Salamini, F. Variability of essential amino acid content in seeds of 22 *Phaseolus* species. *Theor. Appl. Genet.* **1973**, *43*, 75–78.
(40) Evans, I. M.; Boulter, D. *S*-Methyl-L-cysteine content of various legume meals. *Plant Foods Hum. Nutr.* **1975**, *24*, 257–261.
(41) Kasai, T.; Shiroshita, Y.; Sakamura, S. gamma-Glutamyl peptides of *Vigna radiata* seeds. *Phytochemistry* **1986**, *25*, 679–682.
(42) Delatorre, P.; Rocha, B. A.; Souza, E. P.; Oliveira, T. M.; Bezerra, G. A.; Moreno, F. B.; Freitas, B. T.; Santi-Gadelha, T.; Sampaio, A. H.; Azevedo, W. F., Jr.; Cavada, B. S. Structure of a lectin from Canavalia gladiata seeds: New structural insights for old molecules. *BMC Struct. Biol.* **2007**, *7*, 52.
(43) Humayun, M. A.; Turner, J. M.; Elango, R.; Rafii, M.; Langos, V.; Ball, R. O.; Pencharz, P. B. Minimum methionine requirement and cysteine sparing of methionine in healthy school-age children. *Am. J. Clin. Nutr.* **2006**, *84*, 1080–1085.
(44) Boutrif, E. Recent developments in protein quality evaluation. Food Nutr. Agric. [Online] 1991; FAO Document Repository http://www.fao.org/DOCREP/U5900T/U5900T07.HTM (accessed October 26, 2007).
(45) Badaloo, A.; Reid, M.; Forrester, T.; Heird, W. C.; Jahoor, F. Cysteine supplementation improves the erythrocyte glutathione synthesis rate in children with severe edematous malnutrition. *Am. J. Clin. Nutr.* **2002**, *76*, 646–652.
(46) Tor-Agbidye, J.; Palmer, V. S.; Lasarev, M. R.; Craig, A. M.; Blythe, L. L.; Sabri, M. I.; Spencer, P. S. Bioactivation of cyanide to cyanate in sulfur amino acid deficiency: relevance to neurological disease in humans subsisting on cassava. *Toxicol. Sci.* **1999**, *50*, 228–235.
(47) Neuhierl, B.; Bock, A. On the mechanism of selenium tolerance in selenium-accumulating plants. Purification and characterization of a specific selenocysteine methyltransferase from cultured cells of *Astragalus bisulcatus*. *Eur. J. Biochem.* **1996**, *239*, 235–238.
(48) Ellis, D. R.; Sors, T. G.; Brunk, D. G.; Albrecht, C.; Orser, C.; Lahner, B.; Wood, K. V.; Harris, H. H.; Pickering, I. J.; Salt, D. E. Production of *Se*-methylselenocysteine in transgenic plants expressing selenocysteine methyltransferase. *BMC Plant Biol.* **2004**, *4*, 1.
(49) Lyi, S. M.; Heller, L. I.; Rutzke, M.; Welch, R. M.; Kochian, L. V.; Li, L. Molecular and biochemical characterization of the selenocysteine *Se*-methyltransferase gene and *Se*-methylselenocysteine synthesis in broccoli. *Plant Physiol.* **2005**, *138*, 409–420.
(50) Benevenga, N. J.; Case, G. L.; Steele, R. D. Occurence and metabolism of *S*-methyl-L-cysteine and *S*-methyl-cysteine sulfoxide in plants and their toxicity and metabolism in animals. In *Toxicants of Plant Origin*; Cheeke, P. R. Ed.; CRC Press Inc.: Boca Raton, FL, 1989; Vol. III, pp 203−228.
(51) Leustek, T.; Martin, M. N.; Bick, J. A.; Davies, J. P. Pathways and regulation of sulfur metabolism revealed through molecular and genetic studies. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **2000**, *51*, 141–165.
(52) Rebeillé, F.; Jabrin, S.; Bligny, R.; Loizeau, K.; Gambonnet, B.; Van Wilder, V.; Douce, R.; Ravanel, S. Methionine catabolism in Arabidopsis cells is initiated by a gamma-cleavage process and leads to *S*-methylcysteine and isoleucine syntheses. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 15687–15692.
(53) Goyer, A.; Collakova, E.; Shachar-Hill, Y.; Hanson, A. D. Functional characterization of a methionine gamma-lyase in Arabidopsis and its implication in an alternative to the reverse trans-sulfuration pathway. *Plant Cell Physiol.* **2007**, *48*, 232–242.

**Received for review March 13, 2008. Revised manuscript received May 14, 2008. Accepted May 23, 2008. This work was supported in part by the Ontario Bean Producers' Marketing Board, through Grant 8830 of the Agricultural Adaptation Council, Canada-Ontario Research and Development Program IV, funded by the Ontario Ministry of Agriculture, Food and Rural Affairs and Agriculture and Agri-Food Canada.**

JF800787Y