JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Enhancement of the Primary Flavor Compound Methional in Potato by Increasing the Level of Soluble Methionine

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The primary flavor compound in potato, methional, is synthesized from methionine by the Strecker degradation reaction. A major problem associated with potato processing is the loss of methional. Methional or its precursor, methionine, is not added back during potato processing due to high costs of production. A novel approach to enhance the methional level in processed potato would be to increase the production of its precursor, soluble methionine (Met). Cystathionine γ-synthase (CGS) is a key enzyme regulating methionine biosynthesis in plants. To increase the level of soluble methionine in potato, *Arabidopsis thaliana* CGS cDNA was introduced under transcriptional control of the cauliflower mosaic virus 35S promoter into Russet Burbank potato by *Agrobacterium*-mediated transformation. Ten different transgenic potato lines (CGS1−10) were analyzed. Immunoblot analysis demonstrated that *Arabidopsis* CGS is expressed in the leaves, tubers, and roots of transgenic potato plants. CGS enzymatic activity was higher in the leaves and roots of the transgenic potato lines compared to the wild-type potato. Methionine levels in the leaves, roots and tubers of transgenic potato lines were enhanced as high as 6-fold compared to those in wild type potato plants. The methional level in baked tubers of field-grown transgenic potato lines was increased between 2.4- and 4.4-fold in lines CGS1, CGS2, and CGS4. The increase observed in methional levels correlated with the soluble methionine level in the tubers from the same lines measured before processing. These results provide the first evidence that the methional level can be enhanced in processed potatoes by increasing the production of its precursor, methionine.

KEYWORDS: Methional; methionine; cystathionine γ-synthase; genetically engineered potato

INTRODUCTION

Methional is the characteristic flavor compound responsible for the particular aroma of baked potatoes (*1*). Methional is formed by the Strecker degradation reaction, which involves interaction of α-dicarbonyl compounds that are the intermediate product of the Maillard reaction with methionine (Met) (*1*). A large percentage of methional is lost during potato processing because it is heat labile and readily decomposes to methanethiol, which oxidizes to dimethyl disulfide. Furthermore, derivatives of methionine, such as *S*-methylmethionine, release dimethyl sulfide, which is responsible for the aromas of fish, canned sweet corn, tomato juice, and stewed oysters and clams (*1*). Due to high costs of production, this flavor compound or its precursor, methionine, is not added back during food processing. A novel approach to enhance flavor stability and quality in processed foods would be to increase methional levels in potato by increasing the production of its precursor, soluble methionine (Met).

Cystathionine γ-synthase (CGS) is suggested to be the key enzyme regulating the Met biosynthesis pathway in plants (*2*, *3*). CGS catalyzes the committing step in Met synthesis, which occurs when *O*-phosphohomoserine (OPH) condenses with the thiol group of Cys to form cystathionine (**Figure 1**). Cystathionine, the immediate precursor for methionine, is then cleaved to form homocysteine, which is methylated to form Met. All of these reactions except the final methylation reaction occur in the chloroplast. Methylation of homocysteine occurs in the cytosol. Met is converted to *S*-methylmethionine (SMM) and *S*-adenosyl-L-methionine (SAM). The *Arabidopsis thaliana* CGS gene, which was previously cloned, encodes a 563 amino acid, 60 kDa protein and is interrupted by 10 introns (*4*). The enzymatic activity of CGS is regulated by Met in several plants (*5*, *6*). In *Arabidopsis*, this regulation is at the level of mRNA stability. An *Arabidopsis* mutant, *mto1*, was isolated on the basis of selection for resistance to ethionine, a toxic analogue of Met. The *mto1* mutation caused 10−40-fold overaccumulation of

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10.1021/jf030148c CCC: $25.00
Published on Web 08/14/2003

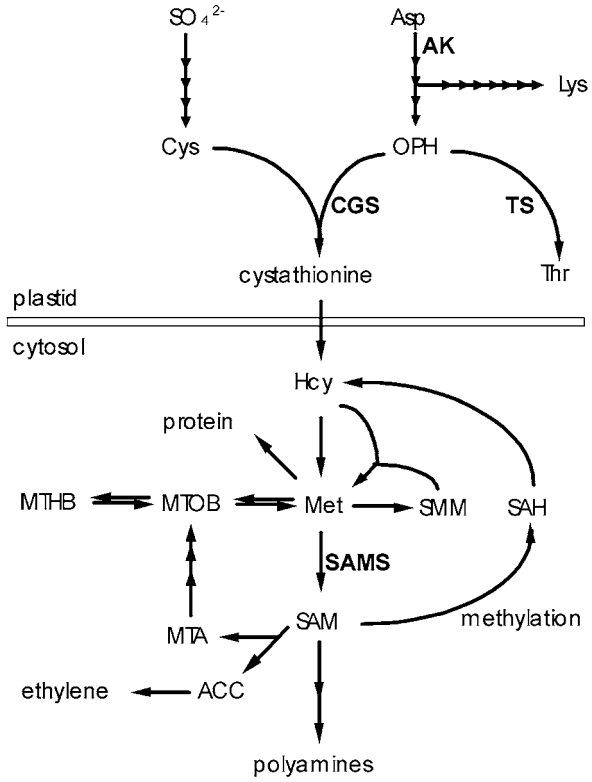

Figure 1. Pathway for Met synthesis and metabolism in plants. The pathway is as described in the Introduction. Dashed arrows represent parts of the pathway with detailed descriptions omitted. Asp, aspartate; Cys, cysteine; Lys, lysine; OPH, *O*-phosphohomoserine; Thr, threonine; Hcy, homocysteine; Met, methionine; SMM, *S*-methylmethionine; SAM, *S*-adenosylmethionine; AK, aspartate kinase; TS, threonine synthase; CGS, cystathionine γ-synthase; SAMS, SAM synthetase.

soluble Met resulting from CGS overexpression (*7*). In wild type *Arabidopsis*, Met or a metabolite of Met down-regulates CGS expression by a post-transcriptional mechanism that destabilizes CGS mRNA. In *mto1*, a point mutation in exon 1 of CGS abolished the Met-dependent destabilization of CGS mRNA, causing the mRNA and protein to accumulate (*8*). The *mto1* mutant overaccumulated Met in the aerial parts during the vegetative growth period; however, the level declined markedly in leaves when the plant entered the reproductive stage of growth (*7*).

Kim et al. (*9*) overexpressed the *Arabidopsis* CGS gene in transgenic *Arabidopsis* plants and showed that soluble Met and SMM levels were increased only at specific stages of development. The accumulation of soluble SMM was even higher than was the accumulation of Met. The highest levels of Met and SMM were observed in the flowers, siliques, and roots of mature transgenic plants, but not in the leaves or other tissues (*9*). These results suggested that in reproductive stage plants, overaccumulated Met in the leaves might be transported into sink tissues (*9*).

In this study, we have overexpressed *Arabidopsis* CGS in Russet Burbank potato plants to determine if soluble Met and methional levels can be enhanced in potato. Our results demonstrate that overexpression of *Arabidopsis* CGS leads to accumulation of soluble Met in the leaves, roots, and tubers of the transgenic potato plants. We show that the methional level is increased after processing of potatoes that accumulate elevated free Met. These results provide the first evidence that enhanced flavor stability and quality can be achieved in processed potatoes by increasing the production of the flavor precursor, free methionine.

EXPERIMENTAL PROCEDURES

Construction of Plant Transformation Vector with CGS. The 2.0 kb *Kpn*I−*Xba*I fragment containing the full-length CGS cDNA clone was first subcloned into pFF19 (*9*). A 3.0 kb *Hind*III−*Eco*RI fragment containing the full-length CGS and the CaMV 35S promoter was cloned into the *Hind*III−*Eco*RI sites of pBI101 (Clontech Laboratories, Palo Alto, CA) in the sense orientation to generate pBI−CGS (*9*). The pBI−CGS was electroporated into the GV2260 strain of *Agrobacterium tumefaciens*, which was used to transform potato.

Transformation and Regeneration of Russet Burbank Potato Plants with *Arabidopsis* CGS. *A. tumefacien*s containing the plant transformation vector, pBI−CGS, was used to transform potato (*Solanum tuberosum* cv. Russet Burbank) stem sections as previously described (*10, 11*).

Southern and Northern Blot Analyses. Genomic DNA was isolated from potato plants by grinding tissues in liquid nitrogen followed by extraction with urea buffer (2.8 M urea, 0.125 M NaCl, 20 mM Tris-HCl, pH 8.0, 8 mM EDTA, 0.4% sarkosyl). After extraction with phenol/chloroform, genomic DNA was precipitated with isopropyl alcohol, and the pellet was resuspended in TE buffer (*12*). Total RNA was isolated using Tri-Reagent (T-9424, Sigma, St. Louis, MO) according to the manufacturer's instructions. Potato tissues were homogenized in Tri-Reagent. Following extraction with chloroform, the total RNAs were precipitated with 2-propanol. Southern and Northern blot analyses were carried out as described by Sambrook et al. (*13*).

Immunoblot Analysis. Potato tissues were homogenized in phosphate-buffered saline, and protein concentrations were determined using the Bio-Rad protein assay reagent (no. 500-0006) with bovine serum albumin as the standard. Ten micrograms of total protein from each sample was resolved on a 10% SDS-PAGE gel in a Mini-Protean II electrophoresis cell (Bio-Rad Laboratories, Hercules, CA). Proteins were electroblotted onto nitrocellulose membrane (no. 162-0112, Bio-Rad) and reacted with an *Arabidopsis* CGS-specific antibody (*9*) followed by peroxidase-conjugated rabbit IgG. The immunoreactive proteins were detected by the enhanced chemiluminescence method (Western Lightning, Perkin-Elmer Life Sciences, Boston, MA).

Methionine and SMM Analysis. Potato leaf samples were collected from the greenhouse-grown plants. Root samples were collected from cuttings growing in flasks containing 50 mL of sterile maintenance medium (*11*). Tubers were sampled from the greenhouse- and field-grown potato plants. Plant samples were ground in liquid nitrogen, and the soluble amino acids from the plant powder were extracted with 20 mM HCl. The extracts were centrifuged for 10 min at 14000*g* to pellet insoluble plant material. Soluble amino acids in the supernatants were derivatized with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) as previously described (*9*). AQC was obtained as part of an AccQ-Fluor reagent kit (Waters, Inc., Milford, MA). Derivatized amino acids were resolved on a 3.9 × 150 mm Waters AccQ-Tag column (Nova-Pak C_{18}) interfaced with a Beckman model 126 solvent delivery system, autosampler, and 32 Karat System Gold data collection and analysis software (Beckman-Coulter, Fullerton, CA) with chromatographic conditions as previously described (*9*). AQC-derivatized amino acids were detected using a Hitachi model F1080 fluorescence detector (Danbury, CT) with an excitation wavelength of 250 nm and an emission wavelength of 395 nm.

CGS Enzymatic Assay. Potato tissue was frozen in liquid nitrogen and ground to a fine powder. The tissue was resuspended in buffer containing MOPS−NaOH, pH 7.4, and then centrifuged at 6000*g* for 10 min. The protein extract was used to measure CGS activity as described in ref *3*. The reaction mixture contained 20 mM Mops−NaOH, pH 7.4, 0.5 mM Cys, 20 mM *O*-succinylhomoserine (OSH), 1 mM DTT, and protein extract in a volume of 100 μL. The reaction was initiated by adding cystathionine and was incubated at 24 °C for 2−30 min. The reaction was stopped after incubation by the addition of 50 μL of 20% (w/v) TCA. Cystathionine in the supernatant was

determined by HPLC after derivatization with *O*-phthalaldehyde (OPA) in the presence of β-mercaptoethanol. A sample of 20 μL was injected into an LiChroCART 250-4 LiChrosorb RP-8 column (Merck) connected to a Beckman model 126 solvent delivery system interfaced with 32 Karat System Gold data collection and analysis software (Beckman-Coulter). Mobile phases used for the elution of the OPA fluorescent derivatives were as follows: mobile A, 85 mM sodium acetate, pH 4.5, containing 6% (v/v) acetonitrile; mobile B, 60% (v/v) acetonitrile. The following linear gradients were used: 40−80% B, 0−6 min; 80% B, 6−9 min; 80−40% B, 9−10 min; 40% B, 10−12 min (40 °C, 1 mL/min). The OPA derivatives were detected by measuring the fluorescence at 455 nm after excitation at 340 nm, using an F-1040 fluorescence detector (Hitachi). Cystathionine was quantified by measuring the peak area. One unit of activity corresponded to the amount of enzyme that catalyzed the formation of 1 μmol of cystathonine per minute.

Field Growth of Transgenic Potatoes. Five selected transgenic potato lines and the wild type potato plants were grown in a field at the Rutgers Agricultural Research and Extension Center. Between 25 and 50 plants for each line were planted in a single plot with 21-ft-long rows and 12-in. spaces between plants. Potato tubers were harvested and stored for 2 months at 4 °C before the analysis.

Methional Analysis. Two months after the harvest, whole field-grown potato tubers (300−400 g) were wrapped in aluminum foil and baked at 205 °C for 60 min. The baked tubers were rapidly cooled to 40 °C in a refrigerator and then chopped and placed in a 1 L flask. The chopped potatoes were spiked with 20 μL of an internal standard solution, 0.0352 g of 1,2-dichlorobenzene/200 mL of methylene chloride, and extracted with methylene chloride (400 and 250 mL successively) for 3 h. The extraction was repeated twice, 3 h each time to allow enough time for the solvent to penetrate the starch matrix and extract the methional. The methylene chloride fraction was collected through filtration. To isolate the volatiles, the collected solvent was applied to a Likens−Nickerson distillation extraction apparatus (*14*) with refluxing in a 1 L round-bottom flask with water (100 mL) for 1.5 h. The isolated volatiles and solvent from the Likens−Nickerson apparatus were dried over Na_2SO_4 and concentrated at 50 °C in a Kuderna−Danish apparatus fitted with a three-ball Snyder column until a final volume of 10 mL was reached. The extract was then slowly concentrated further under nitrogen to a final volume of 0.3 mL. The samples were stored at −20 °C until analyzed. Gas chromatography (GC) was performed on an Agilent 6850 equipped with a flame ionization detector (FID), a 7683 autoinjector, and a fused silica capillary column (30 m, 250 μm i.d., 0.20 μm film thickness, SP-2330, Supelco Inc., Bellefonte, PA). The column temperature was programmed from 50 to 240 °C at a rate of 5 °C/min and held for 5 min at 240 °C. The injector temperature and FID temperature were set at 250 and 300 °C, respectively. A split ratio of 1:1 was used, and the injection volume was 1 μL. The methional was quantified by comparing its GC peak area with that of the internal standard.

Effect of Ethionine on the Transgenic Potato Plants. Ethionine at different concentrations was incorporated into the potato maintenance medium (PM) in test tubes. Cuttings of potato were made with a razor blade, transferred into the tubes containing ethionine, and incubated in the growth chamber. The growth of these potato cuttings was monitored on a daily basis.

RESULTS

Analysis of Transgenic Russet Burbank Potato Lines with Altered Levels of CGS. Russet Burbank potato plants were transformed with *A. tumefaciens* carrying the *Arabidopsis* CGS gene under the control of the 35S promoter. Ten different transgenic lines were generated. All transgenic lines were phenotypically normal and indistinguishable from the untransformed potato plants. The genomic DNA from the transgenic lines was digested with *Kpn*I and *Xba*I to release the 2.0 kb CGS cDNA insert and subjected to Southern blot analysis with ^{32}P-labeled *Arabidopsis* CGS DNA. As shown in **Figure 2**, all transgenic lines contained the 2.0 kb *Kpn*I−*Xba*I CGS cDNA fragment, which was also observed in the genomic DNA from transgenic *Arabidopsis* transformed with the same CGS construct, indicating that all lines are transformed. The *Arabidopsis* CGS cDNA insert did not hybridize to the DNA from the wild type potato plants (wt), demonstrating that the *Arabidopsis* CGS can be distinguished from the endogenous potato CGS. This is not surprising because the *Arabidopsis* CGS protein sequence is only 71.7% identical to potato CGS1 and 70.2% identical to potato CGS2. Southern blot analysis with genomic DNA digested with *Kpn*I revealed that five transgenic lines, CGS1, CGS2, CGS4, CGS8, and CGS10, are independent transformants (data not shown). These lines were chosen for further analysis.

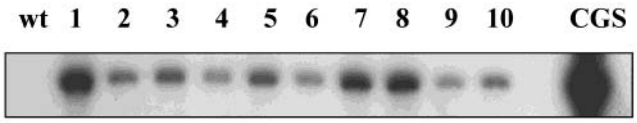

Figure 2. Southern blot analysis of DNA from transgenic potato plants. Genomic DNAs were isolated and purified from wild type (wt) and transgenic potato plants. Genomic DNA from CGS transgenic *Arabidopsis* was included as a positive control (CGS). The DNAs were digested by restriction enzymes *Kpn*I and *Xba*I to release the transgene *Arabidopsis* CGS. The digested genomic DNAs were separated on agarose gel, transferred to nitrocellulose membrane, and hybridized to ^{32}P-labeled CGS probe.

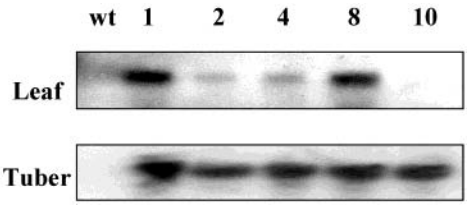

Figure 3. Northern blot analysis of transgenic potato plants. Total RNA isolated from the leaves and tubers of transgenic and wild type (wt) potato plants was separated on a 1.3% agarose gel containing formaldehyde and probed with *Arabidopsis* CGS cDNA insert. Lanes 1, 2, 4, 8, and 10 correspond to transgenic plants CGS1, CGS2, CGS4, CGS8, and CGS10, respectively.

Northern blot analysis indicated that *Arabidopsis* CGS mRNA is expressed in the leaves and tubers of transgenic potato plants (**Figure 3**). *Arabidopsis* CGS cDNA probe did not cross-hybridize to the endogenous potato CGS mRNA in the leaves or tubers. Transgenic lines CGS1 and CGS8 had the highest levels of mRNA in their leaves. CGS1 had higher levels of mRNA in its tubers, while other lines contained similar levels of *Arabidopsis* CGS mRNA in their tubers.

Immunoblot analysis using antibodies specific for *Arabidopsis* CGS demonstrated that the *Arabidopsis* CGS is expressed in the leaves, tubers, and roots of the different transgenic potato lines (**Figure 4**). Polyclonal antibodies against *Arabidopsis* CGS reacted with the purified protein from *Arabidopsis* included as a standard (CGS) in **Figure 4**, but did not cross-react with the potato CGS (wt). Transgenic lines CGS1 and CGS8, which had the highest levels of CGS mRNA in their leaves (**Figure 3**), expressed the highest levels of CGS protein in their leaves (**Figure 4**). CGS1 contained higher levels of CGS mRNA and protein in its tubers (**Figures 3** and **4**). Similar levels of CGS mRNA (**Figure 3**) and protein (**Figure 4**) accumulated in the roots and tubers of the other transgenic lines.

Activity of *Arabidopsis* CGS in Transgenic Potato Lines. The total CGS activity was analyzed in the leaves and roots of transgenic potato plants. The results (**Table 1**) showed that CGS activity was elevated in the leaves and roots of transgenic potato

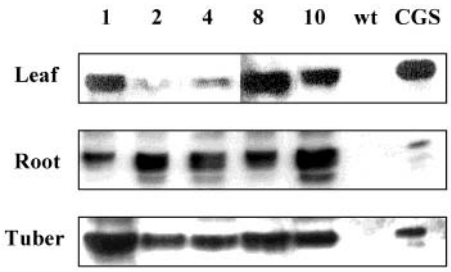

Figure 4. Immunoblot analysis of transgenic potato plants. Protein samples were extracted from potato tissues with phosphate-buffered saline and electrophoresed on 10% SDS-PAGE gel. They were transblotted onto nitrocellulose membrane and probed with *Arabidopsis* CGS polyclonal antibody. Purified CGS protein from CGS-expressing bacteria was used as a positive control (CGS). Lanes 1, 2, 4, 8, and 10 correspond to transgenic plants CGS1, CGS2, CGS4, CGS8, and CGS10, respectively. Wt corresponds to untransformed Russet Burbank potato.

Table 1. CGS Enzymatic Activity in Leaves and Roots of Transgenic Potato Lines Overexpressing *Arabidopsis* CGS

line		specific activity[a] (nmol/min/mg)
wild-type leaf		0.52 ± 0.18
CGS transgenic leaf	CGS1	3.48 ± 0.96
	CGS2	1.21 ± 0.88
	CGS4	1.01 ± 0.29
	CGS8	2.74 ± 0.23
	CGS10	1.72 ± 0.63
wild-type root		0.70 ± 0.11
CGS transgenic root	CGS1	2.05 ± 0.17
	CGS2	0.90 ± 0.01
	CGS4	1.78 ± 0.02
	CGS8	2.85 ± 0.14
	CGS10	1.45 ± 0.38

[a] Average of two independent experiments.

lines compared to the CGS activity in wild type potato plants. The lines with the highest CGS enzymatic activity in their leaves were CGS1, which had 7-fold higher activity than wild type, and CGS8, which had 5-fold higher activity than the wild type potato plants. The CGS enzymatic activity correlated well with the CGS protein levels in the leaves of the same plants (**Figure 3**). In roots, CGS1 had a 3-fold higher activity and CGS8 had a 4-fold higher activity than the wild type potato plants. The CGS protein levels did not correlate as well with enzymatic activity in the roots, possibly because immunoblot analysis and the enzyme assay were not done on the same tissue. We were not able to analyze CGS activity in the tubers of transgenic plants because the high level of polysaccharides present in the tuber tissue interfered with the assay.

Met and SMM Levels in Transgenic Potato Lines. The Met level in the leaves of transgenic potato plants was increased by as much as 6-fold in CGS1 compared to wild type potato plants (**Figure 5**). The methionine level in the roots of the transgenic plants was also enhanced, with CGS8 at a 3-fold higher level compared with the roots of wild type (**Figure 5**). Transgenic line CGS1, which had the highest enzyme activity in the leaves, contained the highest levels of Met in the leaves, whereas CGS8, which had the highest CGS activity in the roots, contained the highest levels of Met in the roots. The Met level in the tubers of transgenic plants that were grown in the greenhouse (GG) were 2−5-fold higher than the level in the wild type tubers, with CGS1 having the highest level. CGS1 had the highest levels of Met in its leaves and tubers when it was grown in the greenhouse (**Figure 5**). When the Met level was analyzed in the tubers of transgenic lines harvested from the field, two transgenic lines, CGS1 and CGS2, had the highest levels. The Met level in the tubers of these lines was 2−2.9-fold higher than the level in wild type tubers. Tubers from CGS2 grown in the greenhouse accumulated lower Met than when tubers from the same line were grown in the field. The differences in Met level in the tubers from greenhouse- or field-grown plants could have resulted from the differences in growth conditions in the greenhouse versus the field and the response of transgenic lines to these conditions.

As shown in **Figure 5**, the Met level in the tubers of transgenic lines was an order of magnitude higher than the Met level in the leaves of the same lines. Because the CGS protein level was not significantly higher in the tubers than in the leaves, these differences are not likely to be due to increases in CGS expression in the tubers of the transgenic plants. These results suggest that Met synthesized in the leaves of transgenic potato plants may be transported into the tubers. Recent results indicate that Met is converted to *S*-methylmethionine (SMM) in the leaves, translocated as SMM in the phloem, and reconverted into Met in the sink tissues of wheat plants (*15*).

Analysis of SMM levels in transgenic potato plants demonstrated that SMM levels were increased in all of the transgenic potato plants, with CGS1 showing the highest increase in both leaves (11.6-fold) and roots (4.3-fold) (**Figure 6**). However, SMM was barely detectable in the tubers of both wild type and transgenic potato plants. These results suggest that as observed in wheat (*15*), SMM is synthesized in the leaves of transgenic potato plants.

Transgenic Potato Plants That Overexpress Methionine Are Resistant to Ethionine. Ethionine is a toxic Met analogue, and resistance to ethionine can be achieved by overproduction of soluble Met (*9*, *16*). We tested the effects of ethionine on the growth of transgenic potato plants by incorporating ethionine into the maintenance medium. Increasing concentrations of ethionine, ranging from 10 to 300 μM, were initially tested using wild type potato plants. The growth of wild type potato plants was completely inhibited at 50 μM and higher levels ethionine. When transgenic potato plants were grown on 25 and 50 μM ethionine, only CGS4 and CGS8 had limited root growth. As shown in **Figure 5**, CGS4 and CGS8 had the highest levels of Met in their roots. At 10 μM ethionine, all transgenic plants and wild type plants initiated roots in 3 days. As shown in **Figure 7**, the transgenic plants continued to grow after 3 days, whereas growth of the wild type plants was inhibited. These results provide further evidence that the roots of transgenic plants accumulate Met. Furthermore, they suggest that ethionine resistance can be used as a functional tool to identify CGS-overexpressing and Met-overproducing potato lines.

Methional Levels in Transgenic Potato Plants. Volatile methional was extracted from field-grown (FG) potato tubers after baking and analyzed by GC. The results in **Figure 8** show that the methional level in field grown tubers from several transgenic lines were higher than the level observed in the tubers from wild type potato plants. Line CGS2 had the highest level of methional, with an average of 78.84 ng/g of tuber tissue, ~4.4-fold higher than the methional level observed in wild type potato tubers. The methional levels were 2.7- and 2.4-fold higher in lines CGS4 and CGS1, compared to wild type potato, whereas CGS8 and CGS10 did not show any detectable increase in methional level. In the majority of transgenic lines, the methional level correlated with the soluble Met level in the tubers of the same lines grown in the field. Lines CGS1 and CGS2, which

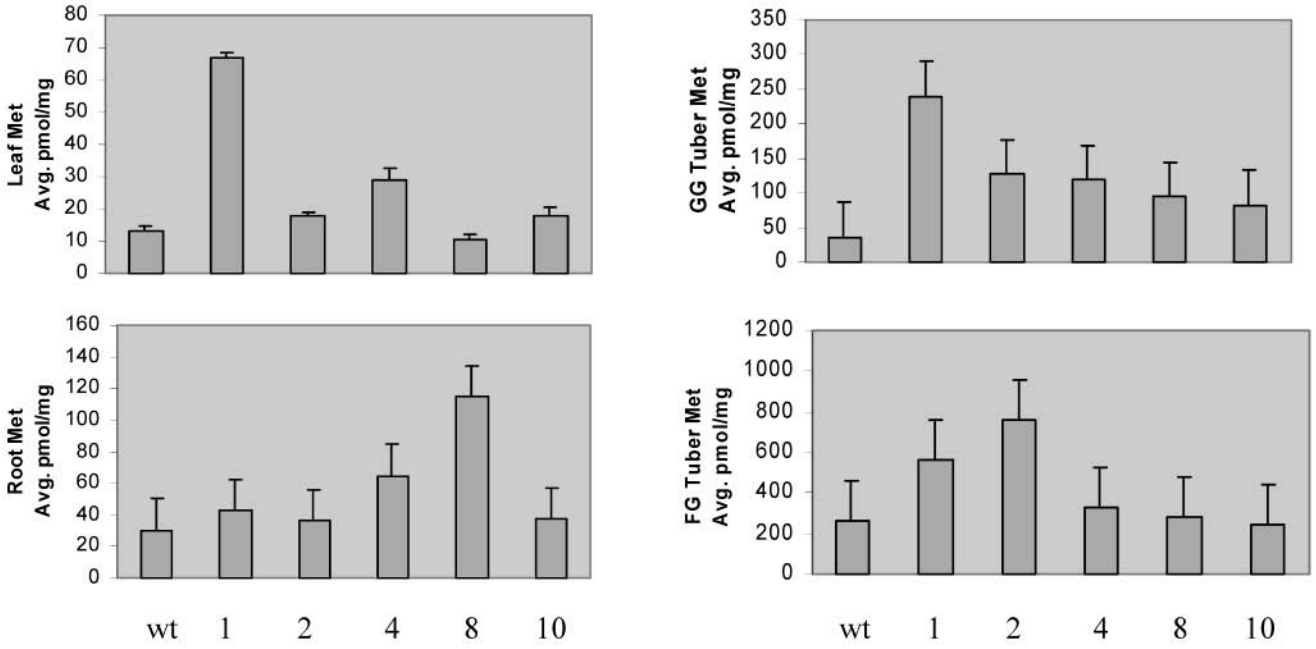

Figure 5. Methionine level in tissues of wild type and CGS-overexpressing transgenic potato plants. Samples from leaves, roots, and tubers were collected as described under Experimental Procedures. Avg., average; GG, greenhouse-grown; FG, field-grown.

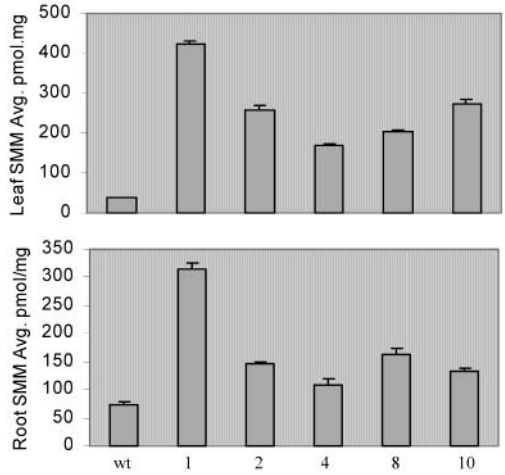

Figure 6. *S*-Methylmethionine level in tissues of wild type and CGS-overexpressing transgenic potato plants. Samples from the leaves and roots of transgenic potato plants were collected, and SMM levels were determined as described under Experimental Procedures. Avg., average.

had the highest levels of soluble Met in their tubers after growth under field conditions, produced higher levels of methional, whereas lines CGS8 and CGS10, which had lower levels of soluble Met, produced lower levels of methional after processing.

DISCUSSION

The results reported here demonstrate that the level of the major flavor compound, methional, can be increased in processed potatoes by increasing the level of its precursor, soluble Met. To increase the level of soluble Met in potato, we introduced the *Arabidopsis* CGS cDNA under transcriptional control of the constitutive cauliflower mosaic virus 35S promoter into Russet Burbank potato. Transgenic potato plants expressing *Arabidopsis* CGS accumulated *Arabidopsis* CGS mRNA and protein and were phenotypically normal. The level of soluble Met was enhanced in the leaves, roots, and tubers of most transgenic lines. The higher level of soluble Met in tubers led to the production of a higher level of the primary flavor compound, methional, after processing. Because the soluble Met level was increased in potato by overexpressing the *Arabidopsis* CGS and even a 2-fold increase in CGS activity was high enough to enhance Met synthesis, these results provide evidence that CGS is the key enzyme regulating Met biosynthesis in potato.

In *Arabidopsis* CGS expression is regulated at the level of the stability of CGS mRNA (*8*). Isolation of an *Arabidopsis* mutant, *mto1*, in which a mutation within the CGS gene increased the stability of the CGS mRNA in the presence of increased levels of Met, provided evidence that CGS is regulated at the post-transcriptional level in *Arabidopsis* (*7*). This mutation led to up to 40-fold increases in Met content in the *mto1* mutant during certain stages of development. During the vegetative growth phase, the level of Met was elevated in the leaves. However, during reproductive growth, the soluble Met level decreased to wild type levels in the leaves and increased by 5−8-fold in the inflorescence and immature fruits of the mutant plants compared to wild type (*7*). These results suggested that the concentration of soluble Met is temporally and spatially regulated in *Arabidopsis* and that soluble Met is translocated into the sink organs after the onset of reproductive growth. Riens et al. (*17*) and Winter et al. (*18*) have shown that the soluble amino acid concentration in phloem sap is proportional to cytosolic concentration, suggesting that Met per se is translocated. Kim et al. (*9*) reported that overexpression of *Arabidopsis* CGS in *Arabidopsis* led to increases in Met levels, but in specific tissues and during specific stages of development. Transgenic *Arabidopsis* plants overexpressing CGS accumulated 8−20-fold higher Met in flowers, siliques, and roots of mature plants, but not in leaves, suggesting that during reproductive growth, overaccumulated Met could be transported from the leaves into the flowers of transgenic *Arabidopsis* plants (*9*). In contrast to these results in *Arabidopsis*, Zeh et al. (*19*) showed that antisense inhibition of threonine synthase (TS) led to increased Met levels in potato and that increased Met content had no detectable effect

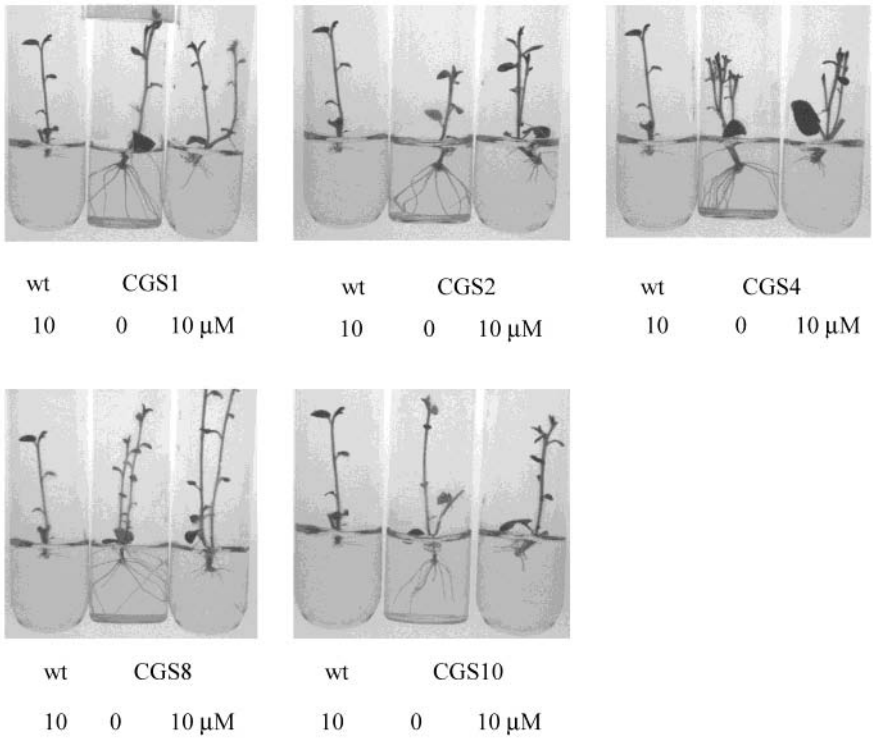

Figure 7. Effect of ethionine on CGS-overexpressing transgenic potato plants. The resistance of transgenic potato plants to the toxic analogue of methionine, ethionine, at the concentration of 10 μM, was compared to that of the wild type (wt) potato. Potato cuttings were grown in maintenance medium (*11*) with ethionine incorporated. The growth of the roots was observed and recorded.

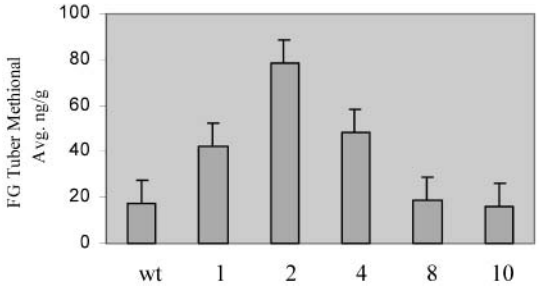

Figure 8. Methional level in potato tubers. Methional was extracted from baked field-grown (FG) wild type (wt) and transgenic potato tubers, resolved by gas chromatography and quantitated as under Experimental Procedures.

on the mRNA or protein level or on the enzymatic activity of CGS in potato.

Our results demonstrate that when *Arabidopsis* CGS is expressed in transgenic potato, CGS protein accumulated and CGS specific activity increased in the leaves of transgenic potato plants. Protein levels in the leaves correlated with the CGS specific activity. Soluble Met accumulated to 6-fold higher levels in the leaves of plants from line CGS1, which had 7-fold higher CGS specific activity than the wild type plants in its leaves. Although the increase in soluble Met content did not correlate with the increase in CGS specific activity in every transgenic line, these results suggest that unlike in *Arabidopsis*, the increased Met level in potato does not down-regulate CGS expression or enzymatic activity and can lead to increased Met levels in the leaves.

The free Met level in greenhouse- or field-grown tubers of the transgenic lines was ~1 order of magnitude higher than it was in the leaves. These results provide evidence that accumulation of Met in potato is regulated in a temporal and special manner. Because Met accumulation in the tubers is greater than the accumulation in the leaves and roots of the same transgenic lines, soluble Met synthesized in the leaves may be translocated to the tubers of the transgenic plants. These results suggest that as in *Arabidopsis* (*7*) a translocation system may operate in potato to maintain the soluble Met concentration in the vegetative organs at a certain level by translocating the soluble Met into the sink organs.

The recent findings of Bourgis et al. (*15*) indicate that SMM is a major amino acid in the phloem and that SMM is mainly synthesized in the leaves from Met, whereas the reconversion from SMM to Met occurs in sink tissues of wheat plants. Our results indicate that SMM levels were elevated in the leaves and roots of transgenic potato plants (**Figure 5**). In contrast, SMM levels were not detectable in the tubers. It has been shown that SMM is essentially absent from mature grains of barley possibly because it is converted to Met (*15*, *20*). Our results provide evidence that SMM is synthesized in the potato source tissues, the leaves, and may be reconverted rapidly to Met in potato sink organs, the tubers. SMM reconversion to Met may occur rapidly in the tubers, because SMM was not detected in the wild type or transgenic potato tubers.

Our results indicate that transgenic potato plants overexpressing CGS are resistant to ethionine, providing further evidence that Met levels are significantly higher in the roots of the transgenic plants. These results indicate that ethionine resistance can be used as a functional tool to identify transgenic potato plants overexpressing CGS and accumulating soluble Met.

The loss of the flavor compound methional during processing causes the food industry significant efforts to restore the desirable flavor to processed foods (*21*, *22*). We have demonstrated that by introducing the *Arabidopsis* CGS gene into potato, we were able to enhance the production of soluble Met and the primary flavor compound, methional, in potato. Previous efforts to overproduce Met using the potato CGS gene were not successful, and the authors concluded that CGS does not

have a role in regulating Met levels in potato (*23*). The lack of overexpression of the potato CGS gene could be due to gene silencing, which has been observed in transgenic plants when multiple copies of a sequence corresponding to an endogenous gene are introduced into the genome (*24*, *25)*. Evidence for gene silencing has been observed in transgenic *Arabidopsis* plants overexpressing *Arabidopsis* CGS (*9*). Silencing of endogenous CGS by expression of either sense or antisense *Arabidopsis* CGS resulted in deformed plants with reduced reproductive growth (*3*, *9*, *26*). Because there is only 70−71% homology between the *Arabidopsis* and potato CGS (*27*, *28*), we did not observe a cross-reaction between the *Arabidopsis* CGS and the endogenous potato CGS and did not obtain any evidence of gene silencing in our transgenic potato plants expressing the heterologous *Arabidopsis* CGS gene. The two cDNAs encoding CGS from potato show 93% similarity in amino acid sequence (*27*, *28*). The N-terminal sequences of the potato CGS cDNAs contain the conserved MTO1 domain in exon 1 that is mutated in *mto1*, which leads to stabilization of CGS expression in *Arabidopsis* (*8*). Thus, if the stability of the potato CGS is regulated by Met levels, Met accumulation may lead to destabilization of the endogenous CGS mRNA in potato.

A direct correlation was observed between the soluble Met level in tubers harvested from the field and the methional level in tubers from the same transgenic lines after processing. These results demonstrate that expression of a heterologous CGS gene leads to elevated soluble Met, which in turn results in enhanced production of the primary flavor compound, methional, in processed potatoes. These findings provide evidence that flavor stability and quality can be enhanced in processed potato products by increasing the production of the flavor precursor, methionine, and suggest that a similar strategy may be developed to enhance the methional content of other processed food crops.

ACKNOWLEDGMENT

We thank Drs. Steve Johnston and Melvin Henninger for help with field growth of potatoes.

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Received for review February 28, 2003. Accepted July 17, 2003. This is publication D10535-1-03 of the New Jersey Agricultural Experiment Station supported by state funds and the Center for Advanced Food Technology (CAFT). This work was supported by a grant from CAFT (to N.E.T.) and the National Science Foundation (Grants MCB-9728661 to T.L. and MCB-0094062 to M.N.M.)

JF030148C