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Effects on GSH Synthesis in Chinese Cabbage When the Culturing Solution Is Supplemented with Ammonium Sulfate or the Constituent Amino Acids for Glutathione

Ju-Sung Kim[1], Sang-Gyu Seo[1], Sun-Hyung Kim[2], Kenji Usui[1], and Ie-Sung Shim[3]*

[1]Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan
[2]Research Institute of Natural Resources, Ishikawa Prefectural University, Suematu, Nonoichi, Ishikawa 921-8836, Japan
[3]Department of Environmental Horticulture, University of Seoul, Seoul 130-743, Korea

We examined the effects of the constituent amino acids for glutathione (GSH) -- glutamate (Glu), cysteine (Cys), and glycine (Gly) -- on GSH synthesis in Chinese cabbage seedlings. Glu, Cys, and Gly were applied simultaneously (100 mg L^{-1}) to the culture solution for 2 d. When compared with the control, GSH concentrations were increased by 2.1-fold (640.4 nmol g^{-1} FW) and 1.5-fold (416.4 nmol g^{-1} FW) in the first leaf and the roots, respectively. Of all the free amino acids, the non-essentials, including Glu, Cys and Gly, occupied 95.5% (shoots) and 81.9% (roots) of the total. Cys supplements greatly enhanced the GSH concentration in the roots; application of 100 mg L^{-1} increased the level by 7-fold over the control. The activity of GSH synthetase was higher in the roots than in the leaf, whereas that of γ-glutamylcysteine synthetase was higher in the leaf.

Keywords: chinese cabbage, cysteine, glutathione, glutathione synthetase, γ-glutamylcysteine synthetase

Chinese cabbage (*Brassica campestris*) is the most important vegetable crop in Korea. The best quality produce contains a high concentration of glutathione (GSH). This component has several functions in plant defense systems, such as an antioxidant involved in the redox balance of cells (Kunert and Foyer, 1993). It is also effective in detoxifying xenobiotica and heavy metals (Rennenberg, 1982; Alscher, 1989), serving as a cofactor for some enzymes and DNA synthesis, and playing a central role in the metabolism of reduced sulfur (Schmidt and Kunert, 1986). Therefore, it is important to study the factors that enhance the capacity to produce or metabolize GSH in plants.

GSH is a tripeptide comprising L-glutamate (Glu), L-cysteine (Cys), and glycine (Gly). It is synthesized from Glu, Cys, and Gly through two consecutive adenosine triphosphate (ATP)-dependent steps. First, dipeptide γ-EC is produced from Glu, and Cys is catalyzed by γ-glutamylcysteine synthetase (E.C. 6.3.2.2, γ-ECS) (Lancaster et al., 1989; Hell and Bergmann, 1990; Steffens, 1990; Ruegsegger and Brunold, 1992). Second, Gly is added to the c-terminal site of the dipeptide (γ-EC) to yield GSH; this reaction is catalyzed by GSH synthetase (E.C. 6.3.2.3, GSHS) (Law and Halliwell, 1986; Klapheck et al., 1987; Macnicol, 1987; Hell and Bergmann, 1988; Ruegsegger et al., 1990).

In Japan, research was active from 1976 to 1985 on the fermentative and enzymatic production of GSH for food additives, supplements, and medicines. The fermentative products of GSH by yeast were commercialized in the early 1980s (Li et al., 2004). Much of the technology for GSH production has since been patented. Several materials have a stimulatory effect, e.g., wastewater in yeast (Asai and Kume, 1943); amino acids in *Lactococcus lactis* (Li et al., 2005), *Rhodotorula glutinis* (Cho et al., 1978), and *Escherichia coli* (Li et al., 1998); anisomycin in *Saccharomyces cerevisiae* (Watanabe, 1973); and amino acids in *S. cerevisiae* (Kusakabe, 1973; Mimura, 1973; Ooka, 1973a, b; Wen et al., 2004).

The underlying control mechanisms leading to up-regulation of GSH synthesis in plants have not been fully defined. Therefore, to elucidate the effect of additives, such as constituent amino acids, on GSH production in Chinese cabbage, we examined how the concentrations of GSH, γ-EC, and cysteine in seedlings were influenced when hydroponic culture solutions were supplemented with either constituent

*Corresponding author; fax +82-2-2210-2838
e-mail isshim@uos.ac.kr

Abbreviations: EAA, essential amino acid; GSH, glutathione; GSHS, glutathione synthetase; NEAA, non-essential amino acid; TAA, total amino acids; γ-ECS, γ-glutamylcysteine synthetase

amino acids of GSH or ammonium sulfate.

MATERIALS AND METHODS

Plant Material and Treatment

Seeds of Chinese cabbage (*B. campestris* cv. Taiby-ourokujunichi) were germinated and grown for 7 d in cell flats (3 cm×3 cm×10 cm) filled with vermiculite. Afterward, the flats were transferred to a cultivation chamber for growth under controlled environmental conditions, including a relative humidity of 60 to 80%, 25°C/15°C (day/night), and a 16-h photoperiod. The culture solution comprised (in mg L^{-1}) 40 N (NO_3^-: NH_4^+ =1:1), 40 P_2O_5, 40 CaO, 10 MgO, 5 Fe_2O_3, 0.5 MnO, 0.4 B, 0.05 Mo, 0.02 Cu, and 0.05 Zn, as described by Ohta et al. (1970). The nutrient solution (pH 5.5 to 6.0) was renewed every 2 d and was aerated continuously. Uniform seedlings (7 d old) were transplanted to this nutrient solution in 2.9-L aerated tanks, and were grown until the second leaf had fully expanded (7 d after transfer). Ammonium sulfate or a constituent amino acid of GSH (Glu, Cys, and Gly), either alone or in combination, was then added, at concentrations of 0 (control), 50, or 100 mg L^{-1}. Two days later, the seedlings were harvested, and their roots and first leaf were separated for immediate storage in liquid nitrogen at -80°C.

Extraction and Preparation of Thiol Derivatives of GSH, γ-EC, and Cys

GSH, γ-EC, and Cys in the plant extracts were converted to thiol derivatives as described by Kocsy et al. (2000). The plant material was ground with liquid nitrogen in a mortar, then 5 mL of 0.1 M HCl containing 1 mM Na_2EDTA was added to 500 mg of the sample. After mixing, the solution was centrifuged at 16,000g for 15 min at 4°C. Then, 400 μL of the supernatant was added to 600 μL of 0.2 M 2-[cyclohexylamino] ethane sulphonic acid (pH 9.3), and reduced with 100 μL of a freshly prepared 400 mM $NaBH_4$ solution. The mixture was kept on ice for 20 min, and 15 μL of 15 mM monobromobimane (Fahey and Newton, 1987) was added to 330 μL of the mixture. Afterward, the mixture was kept in the dark at room temperature for 15 min to obtain the thiol derivatives. This reaction was stopped by adding 250 μL of 5% (v/v) acetic acid, and the reactant was centrifuged at 16,000g for 15 min at 4°C.

Determination of Free Amino Acids

Free amino acids were extracted according to the method of Desmaison et al. (1984), with minor modifications. Frozen tissues were ground to a fine powder in liquid nitrogen with a chilled mortar and a pestle, then 300 to 500 mg of the sample was homogenized in 2 mL of 15 mM HCl. The homogenate was centrifuged at 2,000g for 5 min at 4°C, and 500 μL of the supernatant was de-proteinized with 100 μL of 5-sulfosalicylic acid (10% w/v) and kept on ice for 15 min. The mixture was again centrifuged at 2,000g for 15 min at 4°C, and the supernatant (500 μL) was collected and adjusted to pH 2.2 to 2.3. The concentrations of free amino acids were measured with an amino acid analyzer (JLC-300, JEOL; Japan) and compared with standard amino acid solutions (AN and B types; Wako Pure Chemicals, Japan).

Assay of γ-ECS and GSHS Activities

Activities of γ-ECS and GSHS were assayed via the method described by Hell and Bergmann (1990), with some modification. In a chilled mortar, 0.5g of the frozen samples was ground with 10 mL of extraction buffer [0.1 M Tris-HCl (pH 7.5) and 5 mM EDTA] and 1 g of polyvinylpolypyrrolidone (Sigma, USA). This slurry was centrifuged at 15,000g for 15 min at 4°C, and the supernatant was used to determine activity.

To assay for γ-ECS, a mixture of 100 mM Tris-HCl (pH 7.5), 50 mM $MgCl_2$, 1 mM DTT, 10 mM ATP, 50 mM Na-L-glutamate, and 285 μL of protein extract (total volume of 500 μL) was pre-incubated in a tube at 37°C for 10 min, then 2 mM L-cysteine (Cys) was added. After another 45 min of incubation, the reaction was stopped by adding 50 μL of 50% (w/v) 5-sulfosalicylic acid, and the tube was transferred to ice. Denatured proteins were removed by centrifugation and the supernatants were assayed for γ-ECS via HPLC. The quantity of γ-ECS synthesized was determined by comparison with the peak area for authentic γ-EC (Nacalai Tesque, Japan). As the control, a mixture without additional Cys was also assayed.

For the GSHS assay, a mixture of 100 mM Tris-HCl (pH 8.0), 50 mM KCl, 10 mM $MgCl_2$, 5 mM ATP, 5 mM creatine phosphate, 2 units of creatine kinase, 1.5 mM γ-EC, and 295 μL of protein extract (total volume of 500 μL) was pre-incubated at 30°C for 10 min, then 10 mM glycine (Gly) was added. After another 30 min of incubation, the reaction was terminated and subjected to HPLC analysis as described

for the γ-ECS assay. A mixture without supplemental Gly served as the control.

Protein Content Assay

Protein content was measured in the first leaf and root extracts using the standard Biorad Coomassie brilliant blue assay as described by Bradford (1976).

HPLC of Thiol Derivatives

An LC-6A HPLC system (Shimadzu, Japan) was equipped with an SPD-6A detector, SCL-6A system controller, and C-R8A data module. It also included a 5-micron C_{18} Intersil ODS-2® column 5 (150×4.6 mm; GC Science, Japan). The mobile phase A contained 0.1% TFA in water; mobile phase B was MeOH, complementary to the TFA solution (A%= 100%-B%). A gradient elution using MeOH was performed for better separation, and the column was cleansed between subsequent injections. For analysis of GSH, γ-EC, and cysteine, the mobile phase system was programmed as follows: 0 to 30 min, 15 to 22% B; 30 to 35 min, 22 to 100% B; 35 to 40 min, isocratic 100% B; 40 to 45 min, 100 to 15% B; 45 to 50 min, isocratic 15% B. The chromatograph was operated at 30, with a flow rate of 0.8 mL min^{-1}. The fluorescence detector was set at 380 nm for excitation and 480 nm for the emission mode.

Chemicals

The following chemical sources were used: glutathione (GSH), glutathione disulfide (GSSG), diethyl ether, Glu, Gly, and Cys, from Wako Pure Chemicals; γ–EC, from Nacalai Tesque (Japan); and monobromobimane (mBBr), from Calbiochem (USA). All other reagents in the study were of the highest quality available.

RESULTS

Effects of Supplemental Constituent Amino Acids on GSH Biosynthesis

Hydroponic culture solutions for *B. campestris* seedlings were supplemented with the constituent amino acids for GSH (Glu, Cys, and Gly) either singly or in combination. Concentrations of Cys, γ-EC, and GSH were examined in the first leaf (hereafter called leaves) and roots. In the control, to which no amino acids were added, leaves concentrations of GSH and γ-EC were 299.5 ± 8.8 and 29.9 ± 1.2 nmol g^{-1} FW,

Table 1. Effects of constituent amino acids (Glu, Cys, and Gly) added to hydroponic culture solution on concentrations of Cys, γ-EC, and GSH in the first leaf and roots of Chinese cabbage. All values are means ± SE of five independent extractions. Concentrations are shown as nmol g^{-1} fresh weight.

		Cys		γ-EC		GSH	
		1st leaf	Root	1st leaf	Root	1st leaf	Root
Control		71.1 ± 5.0	199.8 ± 5.1	29.9 ± 1.2	64.2 ± 3.5	299.5 ± 8.8	286.1 ± 9.4
Glu	50 mg L^{-1}	45.8 ± 2.8	77.9 ± 6.5	17.8 ± 1.6	17.7 ± 1.1	351.7 ± 26.7	487.1 ± 42.9
	100 mg L^{-1}	79.1 ± 4.9	110.2 ± 2.3	15.8 ± 1.4	22.5 ± 0.4	472.0 ± 13.4	607.2 ± 30.9
Cys	50 mg L^{-1}	271.0 ± 23.9	261.0 ± 21.4	245.2 ± 2.7	359.6 ± 16.2	452.2 ± 22.6	960.9 ± 52.2
	100 mg L^{-1}	540.6 ± 6.1	756.1 ± 30.8	266.1 ± 8.1	654.9 ± 25.0	576.5 ± 6.6	1997.2 ± 90.4
Gly	50 mg L^{-1}	78.5 ± 7.3	301.9 ± 6.0	19.5 ± 1.6	63.2 ± 1.2	423.3 ± 23.0	489.2 ± 7.0
	100 mg L^{-1}	75.0 ± 11.5	304.9 ± 6.3	26.5 ± 1.2	69.7 ± 3.2	425.9 ± 36.6	481.0 ± 6.8
Glu+Cys	50 mg L^{-1}	174.7 ± 5.4	359.5 ± 10.1	224.0 ± 10.3	102.9 ± 5.3	404.1 ± 8.4	513.9 ± 20.0
	100 mg L^{-1}	225.4 ± 4.7	371.0 ± 10.9	641.8 ± 7.5	323.8 ± 7.5	392.1 ± 4.9	558.3 ± 3.6
Cys+Gly	50 mg L^{-1}	311.4 ± 5.2	760.8 ± 11.4	482.7 ± 33.7	360.5 ± 12.1	437.0 ± 2.5	522.3 ± 47.5
	100 mg L^{-1}	233.7 ± 1.1	623.4 ± 17.3	199.4 ± 3.9	115.5 ± 1.5	458.9 ± 2.9	559.4 ± 29.4
Glu+Gly	50 mg L^{-1}	48.9 ± 5.4	225.9 ± 2.3	34.3 ± 0.3	104.9 ± 11.1	458.6 ± 33.6	408.4 ± 6.2
	100 mg L^{-1}	35.3 ± 4.2	207.9 ± 1.8	30.6 ± 4.9	107.9 ± 1.5	432.4 ± 28.4	428.0 ± 14.5
Glu+Cys+Gly	50 mg L^{-1}	77.0 ± 5.4	352.4 ± 3.2	42.5 ± 0.3	148.8 ± 7.6	605.0 ± 22.1	380.6 ± 21.7
	100 mg L^{-1}	118.6 ± 4.2	274.6 ± 15.4	112.2 ± 4.9	150.1 ± 3.1	640.4 ± 16.5	416.4 ± 15.0

respectively; root concentrations were 286.1 ± 9.4 and 64.2 ± 3.5 nmol g^{-1} FW, respectively (Table 1).

When Glu, Cys, and Gly were added individually, each increased GSH concentrations in both the leaves and roots; Cys was particularly effective in enhancing root GSH levels (Table 1). Although the concentration of γ-EC was not affected by Glu and Gly, it was greatly increased by Cys.

The combined application of Glu, Cys, and Gly (Glu+Cys+Gly) at 50 or 100 mg L^{-1} elevated leaf GSH concentrations by 2.0- to 2.1-fold over the control. It also increased the root concentrations by 1.3- to 1.5-fold compared with the control (Table 1). Treatments with Glu+Cys, Cys+Gly, and Glu+Gly also raised GSH concentrations in both the leaves and roots. Glu+Cys and Cys+Gly increased the concentrations of Cys and γ-EC in both leaves and roots as single application of Cys did.

Applications of Glu+Cys+Gly at 50 and 100 mg L^{-1} increased the activity of GSHS in the leaves about 1.3-fold and 1.8-fold, respectively, compared with the control; the activity of leaves γ-ECS was about 1.9-fold and 2.3-fold, respectively, higher than the control. This enhanced γ-ECS activity may have accounted for the rise in GSH concentration. Activities of GSHS and γ-ECS in the roots were also increased similarly by Glu+Cys+Gly (Fig. 1).

At the early steps in the sulfur assimilation pathway, exogenous applications of intermediates, such as sulfide and SO_3^{2-}, are toxic to cells (Andreas and Mark, 2002). Thus, we examined the effect of ammonium sulfate $(NH_4)_2SO_4$ on concentrations of Cys, γ-EC, and GSH (Fig. 2). In the leaves, such treatment had

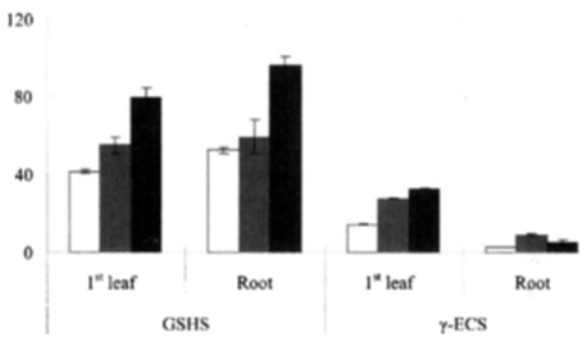

Figure 1. Effect of Glu+Cys+Gly in hydroponic culture solution on GSH synthesis in Chinese cabbage. GSHS and γ-ECS were assayed in extracts from seedlings 2 d after additions. Data are means ± SE of five independent extractions from first leaf and roots. Enzyme activities are shown as nmol min^{-1} g^{-1} protein. White bar: control; gray bar: Glu+ Cys+Gly (50 mg L^{-1}); black bar: Glu+Cys+Gly (100 mg L^{-1}).

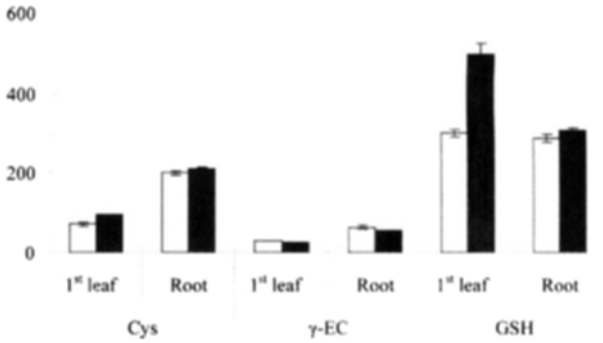

Figure 2. Effect of ammonium sulfate on concentrations of Cys, γ-EC, and GSH from first leaf and roots of Chinese cabbage. 100 mg L^{-1} $(NH_4)_2SO_4$ is equivalent to 0.76 mM. All values are means ± SE of five independent extractions. Enzyme activities are shown as nmol min^{-1} g^{-1} protein. White bar: control; black bar: 100 mg L^{-1} $(NH_4)_2SO_4$.

no significant influence, except at 100 mg L^{-1}, where the level of GSH increased (Fig. 2). Therefore, we infer from this result that ammonium sulfate does not suppress GSH biosynthesis but, rather, promotes it when a high dose is applied.

Effects of Constituent Amino Acids on Concentrations of Free Amino Acids, Urea, and Ammonia in Chinese Cabbage

The shoot concentration of total amino acids (TAA), especially the non-essential amino acids (NEAA), was decreased by supplemental Glu+Cys+Gly (Table 2). In the control seedlings, the level of total essential amino acids (EAA) was only 5.0% of TAA; that of total NEAA occupied 95.0% of TAA. In the shoots of seedlings supplied with Glu+Cys+Gly at 100 mg L^{-1}, NEAA and EAA occupied 95.5% and 4.5% of TAA, respectively. In the shoots, concentrations of methionine and valine (both EAA), and alanine, cystine, glutamic acid, and proline (all NEAA) were increased by 100 mg L^{-1} Glu+Cys+Gly, although the amounts of other amino acids decreased (Table 2).

Likewise, root concentrations of TAA were decreased by the addition of Glu+Cys+Gly, but the concentration of total NEAA, including glutamic acid, and glycine, rose in response to 100 mg L^{-1} Glu+Cys+Gly (Table 2).

Interestingly, NH_3 concentrations were extremely higher in the roots than in the shoots. Britto and Kronzucker (2002) have also demonstrated that the allocation of carbon is higher in the roots than in the shoots for amino acid synthesis under NH_4^+ nutrition. The concentration of ammonia was greatly decreased

Table 2. Concentrations of free amino acids in the shoots and roots of Chinese cabbage supplemented with Glu+Cys+Gly. Protein and free amino concentrations are shown as mg g^{-1} fresh weight and nmol g^{-1} fresh weight, respectively. All values are means ± SE of five independent extractions. n.d.; not detected, E/T; essential amino acid/total amino acid, NE/T; non-essential amino acid/total amino acid.

	Control		Glu+Cys+Gly (50 mg L^{-1})		Glu+Cys+Gly (100 mg L^{-1})	
	Shoot	Root	Shoot	Root	Shoot	Root
Arginine	13.1 ± 3.1	229.4 ± 11.6	11.2 ± 1.0	91.2 ± 3.9	7.1 ± 0.5	78.1 ± 4.5
Histidine	12.7 ± 2.3	281.6 ± 4.2	11.4 ± 0.4	150.1 ± 2.9	9.5 ± 1.5	138.1 ± 3.6
Isoleucine	28.4 ± 2.2	336.9 ± 18.5	28.9 ± 1.7	168.3 ± 2.8	22.5 ± 1.3	124.3 ± 3.9
Leucine	18.2 ± 2.5	282.0 ± 16.4	15.1 ± 0.3	167.1 ± 2.8	13.9 ± 0.5	131.9 ± 3.5
Lysine	22.0 ± 2.7	460.9 ± 26.0	11.1 ± 1.3	211.2 ± 6.0	8.6 ± 0.7	152.1 ± 5.6
Methionine	3.2 ± 0.0	30.6 ± 1.7	4.8 ± 0.5	21.4 ± 1.1	4.2 ± 0.2	20.7 ± 0.9
Phenylalanine	20.7 ± 0.4	64.4 ± 3.0	17.4 ± 1.5	60.3 ± 1.7	17.0 ± 0.7	51.4 ± 1.9
Threonin	116.3 ± 5.8	333.5 ± 13.7	79.0 ± 4.4	212.8 ± 1.7	81.1 ± 3.7	195.8 ± 5.7
Valine	45.7 ± 7.7	637.8 ± 42.3	48.9 ± 2.3	268.7 ± 11.2	49.3 ± 6.6	209.9 ± 5.3
EAA	280.5 ± 26.7	2657.1 ± 137.4	227.9 ± 13.6	1351.2 ± 34.1	213.2 ± 15.7	1102.4 ± 34.8
Alanine	285.8 ± 16.2	107.4 ± 6.5	334.2 ± 14.1	216.6 ± 33.2	296.0 ± 24.1	395.4 ± 22.1
Asparagine	n.d.	11.6 ± 0.3	n.d.	14.0 ± 1.5	n.d.	27.1 ± 2.5
Aspartic acid	1449.7 ± 15.5	549.0 ± 8.1	1583.9 ± 36.1	489.2 ± 26.0	1431.2 ± 62.1	626.6 ± 47.5
Cystine	3.4 ± 0.5	111.5 ± 4.9	9.4 ± 0.7	15.0 ± 2.3	11.7 ± 2.4	16.6 ± 3.4
Glutamic acid	2244.6 ± 49.0	489.0 ± 6.0	1529.9 ± 33.0	618.6 ± 45.4	1704.7 ± 61.6	578.0 ± 46.3
Glutamine	20.9 ± 4.5	69.1 ± 3.4	93.2 ± 8.1	220.0 ± 33.6	67.3 ± 2.5	382.6 ± 40.9
Glycine	16.4 ± 3.4	120.8 ± 6.2	15.8 ± 4.4	170.3 ± 34.6	13.5 ± 0.9	236.8 ± 15.9
Proline	146.0 ± 5.4	43.5 ± 1.5	129.0 ± 4.6	33.0 ± 1.0	151.2 ± 14.0	31.9 ± 1.2
Serine	411.0 ± 31.1	759.8 ± 36.2	201.2 ± 0.6	517.4 ± 46.0	237.1 ± 28.7	567.1 ± 22.5
Tyrosine	12.8 ± 0.9	156.5 ± 6.4	11.1 ± 0.3	101.3 ± 0.9	10.4 ± 0.3	80.4 ± 3.1
NEAA	5336.8 ± 173.1	4915.1 ± 175.8	4650.3 ±139.6	4263.0 ± 331.9	4570.1 ± 239.0	4996.1 ± 284.0
TAA	5617.3 ± 153.1	7572.2 ± 217.0	4878.2 ±115.6	5614.1 ± 258.5	4783.3 ± 212.2	6098.6 ± 240.1
E/T ratio	5.0	35.1	4.7	24.1	4.5	18.1
NE/T ratio	95.0	64.9	95.3	75.9	95.5	81.9
Protein content	3.54 ± 0.3	3.36 ± 0.1	3.92 ± 0.2	2.71 ± 0.1	3.94 ± 0.2	2.86 ± 0.1
Urea	108.9 ± 8.1	1541.6 ± 49.7	108.3 ± 7.1	1075.7 ± 55.9	134.6 ± 17.0	1319.8 ± 45.4
Ammonia	244.1 ± 34.2	9521.5 ± 296.4	295.4 ± 19.7	4552.4 ± 89.0	237.7 ± 24.7	4171.7 ± 71.3

by Glu+Cys+Gly; that of urea, to a lesser degree.

DISCUSSION

Though some environmental conditions, stresss or chemicals have been reported to increase GSH concentration in plants (Hirase and Molin, 2003; Kopriva and Rennenberg, 2004; Tausz et al., 2004). To increase the production of GSH in Chinese cabbage, we added its constituent amino acids -- Glu, Cys, and Gly -- to the culture solution. All three promoted GSH synthesis, a result previously reported with *E. coli* (Li et al., 1998). The combined application of Glu, Cys, and Gly was the most effective in increasing leaf levels of GSH. Moreover, Cys supplied at 100 mg L^{-1} almost doubled this concentration (Table 1). Cys was also extremely effective in increasing root concentrations of GSH; application at 100 mg L^{-1} resulted in a root concentration seven times greater than that measured in the control. This stimulatory effect has also been observed in poplar (Noctor et al., 1996) and in recombinant *E. coli* (Li et al., 1998). In those studies, total GSH and intracellular

GSH concentrations were increased by 40% and 100%, respectively, when 9 mM Cys was added to the culture medium for 12 h. Similar results have been reported by Alfafala et al. (1992).

Factors that promote GSH synthesis also repress the production of γ-EC (Bergmann and Rennenberg, 1993; Schneider and Bergmann, 1995) and Cys (Ruegsegger and Brunold, 1992; Strohm et al., 1995). Here, we also found that leaf concentrations of γ-EC and Cys were as low in seedlings treated with Glu, Gly, Glu+Gly, or Glu+Cys+Gly as those measured in the control, although the GSH concentration clearly was enhanced by the addition of these amino acids. However, supplemental Cys or Gly+Cys markedly increased the levels of Cys and γ-EC (Table 1). Treatment with Cys seemed to stimulate Cys and γ-EC concentrations, although Glu+Cys+Gly had only a slight effect.

A notable characteristic of Chinese cabbage is the high concentration of NH_3 in its roots. Ammonia is mostly formed by the catabolism of protein, where both ingested and cellular proteins are hydrolyzed to form a pool of amino acids that can be used to form new proteins for growth and basic protein turnover (Wright, 1995). In our study, root concentrations of ammonia were decreased dramatically by Glu+Cys+Gly. The concentration of urea was also diminished somewhat by the same treatment (Table 2). Thus, the application of Glu+Cys+Gly increased GSH concentrations while decreasing ammonia toxicity, perhaps due partly to the conversion of ammonia to urea.

In conclusion, the GSH concentration in Chinese cabbage was increased by supplying constituent amino acids (Glu, Cys, and Gly) to the culture solution. Cys application also greatly enhanced root concentrations of GSH. Although more research is necessary, we believe that these additions can improve the nutritional value of this vegetable crop because GSH serves a nutraceutical function in food products.

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