J. Ecol. Field Biol. 29 (1): 61~67, 2006

# Changes of Thiols and Oxidative Stress in Tomato Seedlings Exposed to Cadmium

Cho, Un-Haing* and Nam-Ho Seo
*Department of Biology, Changwon National University, Changwon 641-773, Korea*

**ABSTRACT**: Tomato (*Lycopersicon esculentum* Mill) seedlings exposed to various concentrations of $CdCl_2$ (0~100 $\mu$M) in a nutrient solution for up to 9 days were analyzed with respect to the thiol changes and oxidative stress. The Cd exposure increased total non-protein thiols (NPT) and cysteine in both leaves and roots, total glutathione in leaves, and the ratios of oxidized glutathione (GSSG)/reduced glutathione (GSH) in both leaves and roots, but decreased the ratio of dehydroascorbate (DASA)/ascorbate(ASA) in leaves. Our results suggest that the Cd-induced GSH depletion due to thiol synthesis and oxidation alters the antioxidant activity of seedlings for $H_2O_2$, and the subsequent $H_2O_2$ accumulationand oxidative stress result in phytotoxicity.

***Key words***: Cadmium, Oxidative stress, Thiols, Tomato

## INTRODUCTION

High Cd accumulation generally causes growth inhibition and even death of plants due to the reduction of enzyme activity (Ouariti et al. 1997), photosynthesis (Siedlecka and Baszynski 1993), respiration (Kessler and Brand 1995), transpiration (Barcelo and Poschenrieder 1990) and nutrient uptake (Sanita di Toppi and Gabbrielli 1999).

An increasing body of evidence suggests that the metal-induced phytotoxicity can be attributed, at least in part, to oxidative damage (Dixit et al. 2001, Hegedus et al. 2001, Cho and Sohn 2005). Oxidative stress, arising from an imbalance in the regeneration and removal of reactive oxygen species (ROS) such as hydrogen peroxide ($H_2O_2$), is a challenge faced by all aerobic organisms. In parallel to metal-induced growth inhibition, alterations in both the activities of antioxidant enzymes and the levels of antioxidants as well as enhanced lipid peroxidation have been observed in plants (Weckx and Clijsters 1997, Chaoui et al. 1997, Cho and Park 2000, Schutzendubel et al. 2001, Cho 2004, Cho and Sohn 2005).

Adaptation of the plant cells to metal stress involves chelation, compartmentation or exclusion of metal ions (Mehra and Tripathi 2000). Varying responses to Cd-induced oxidative stress are probably related both to levels of Cd supplied and to concentration of thiols already present, or induced by Cd treatment (Sanita di Toppi and Gabbrielli 1999). Availability of the nonprotein thiols (NPT) including glutathione ($\gamma$-glutamylcysteinyl-gly) and cysteine (Cys) in cytosol may be important since Cd induces the synthesis of phytochelatins (PC, [$\gamma$-glutamylcysteinyl]$_{n=2-11}$-glycines), which bind metals in the cytosol and sequesters them in the vacuole (Grill et al. 1985, Rauser 1995). PCs are the major thiols involved in the physiological mechanism of metal tolerance in various plants (Steffens et al. 1986, Jackson et al. 1987, Salt et al. 1989). The synthesis of the enzymes involved in glutathione formation such as $\gamma$-EC synthetase and glutathione synthetase are induced by exposure to Cd and Cu (Xiang et al. 2001), and might be regulated by the reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio in the cells (Pilon-Smits et al. 2000, Xiang et al. 2001). However, since over-expression of PC synthase and subsequent high PC synthesis led to hypersensitivity to Cd stress in some situations (Lee et al. 2003), and some plants or cell lines resistant to Cd are not active in synthesis of PC (Jackson et al. 1987, Delhaize et al. 1989, Verkleij et al. 1990, Ernst et al. 2000, Ebbs et al. 2002, Schat et al. 2002, Kupper et al. 2004), the role of PC in Cd resistance is not clearly defined. Therefore, whereas PCs may chelate much of the metal in Cd-tolerant cells, it is necessary to recognize that other mechanisms of complexation and physiological amelioration may prevail in plants growing in Cd-polluted soils.

Although PC synthesis induced by Cd-exposure is associated with a rapid depletion of total glutathione (Delhaize et al. 1989, Scheller et al. 1987, Rauser 1987, Ruegsegger et al. 1990, Tukendorf and Rauser 1990), it is still unknown whether the Cd-induced changes in glutathione involve changes in GSH, GSSG, Cys, total NPT and ascorbate. In *Silene cucubalus,* copper caused oxidative stress by depletion of the glutahione due to PC synthesis and a shift in the glutathione redox couple to a more oxidized state (De Vos et al. 1991). However, the Cd ion is unable to catalyze Fenton-Haber-Weiss reactions, which generate ROS. How Cd-exposure

* Corresponding author; Phone: +82-55-279-7445, e-mail: uhcho@sarim.changwon.ac.kr

induces ROS production and oxidative stress is also an interesting subject to investigate.

In this work, the changes of NPTs and ascorbate in tomato seedlings exposed to Cd were investigated to determine if the Cd-induced oxidative stress could be induced by the Cd detoxification process itself, such as PC synthesis and alterations of ROS scavenging systems. PC synthesis and the altered redox status of GSH might have an influence on the GSH-ascorbate cycle and subsequent inefficient quenching of ROS, including $H_2O_2$, might result in oxidative stress leading to phytotoxicity. This information is also pre-required to understand the response of plants to Cd exposure and to develope Cd-resistant plants. The resistant plants might be used in the phytoremediation of Cd-contaminated environment and the re-establishment of ecosystem.

## MATERIALS AND METHODS

### Plant Material and Growth Conditions

Tomato seedlings (*Lycopersic esculentum* Mill cv. Seokwang) were germinated in pots containing a perlite:vermiculite (1:1) mixture in a controlled environment chamber at 25℃ with 12 h of light (250 $\mu$M $m^{-2}s^{-1}$) and 70～80 % humidity. Two week-old seedlings were transferred to aerated nutrient solutions containing half-strength Hoagland's solution (Hoagland and Arnon 1938) with 0, 10, 50 and 100 $\mu$M Cd (as $CdCl_2$). The solution was changed every 3 days. Plants collected from each treatment after 3, 6 and 9 days were dried for 48 h at 70℃ and weighed for biomass, Cd and thiols determination. For measurements of $H_2O_2$ production, fresh samples were weighed and used.

### Determination of Cd Concentration in Seedlings

Leaves were washed twice in deionized water, and the roots of intact plants were washed with ice-cold 5 mM $CaCl_2$ solution for 10 min to displace extracellular Cd (Rauser 1987). The plant material was dried for 48 h at 70℃, weighed and ground into fine powder before wet ashing in $HNO_3$ : $HClO_4$ (3 : 1) solution. Cd was determined directly by atomic absorption spectrophotometry (Varian 200AA equipped with SIPS, Australia) using an air-acetylene flame and Cd hollow-cathode lamp.

### Analyses of Total NPTs, Cys, GSH, GSSG, Dehydroascorbic Acid (DASA) and Ascorbic Acid (ASA)

NPTs were extracted by grinding 20 mg lyophilized leaves or roots in 2 mL 5 %(w/v) sulfosalicylic acid + 6.3 mM diethyllenetriaminepentaacetic acid (pH < 1) at 0℃ and the level of total acid-soluble SH compounds were determined with Ellman's reagent (De Vos et al. 1992). For the determination of GSH and GSSG, 100 mg of samples were powdered in liquid nitrogen, mixed with 1 mL of 5 % (w/v) sulphosalicylic acid and centrifuged (10 min, 4℃, 12,000*g*), and the supernatant was analyzed according to the method of Fadzilla et al. (1997). The level of cysteine was measured according to the method of Gaitonde (1967).

For the extraction of ascorbate, 100 mg of samples were powdered in liquid nitrogen, mixed with 1 mL of 2 % (w/v) metaphosphoric acid containing 1 mM EDTA and 10 mg polyvinylpolypyrrolidone, and centrifuged (20 min, 4℃, 12,000*g*). The supernatant was used for determination of reduced and oxidized ascorbate according to the method of Law et al. (1983). Total ascorbate is determined through a reduction of DASA to ASA by dithiothreitol, and DASA concentrations were deduced from the total ascorbate.

### Statistical Analysis

The results are the means ± SE of five independent replicates. The analyses of variance were computed on statistically significant differences determined by the appropriate F-tests. The mean differences were compared utilizing Duncan's multiple range tests.

## RESULTS AND DISCUSSION

Growth and Cd accumulation were monitored for seedlings grown for 9 d in a nutrient solution containing up to 100 $\mu$M of $CdCl_2$ (Fig. 1). Compared with the control seedlings, the dry weight of seedlings was significantly reduced in the presence of over 50 $\mu$M Cd. The dry weight was reduced by 47 % with 100 $\mu$M Cd, so that seedling growth was sensitive to Cd exposure. Meanwhile, the Cd concentration in all seedlings increased in proportion to the level of treatment applied.

The Cd-induced progressive changes of total NPT, Cys, GSH and the ratio of GSSG/GSH in leaves and roots were measured (Figs. 2～5). Both in leaves and roots, NPT content significantly increased with Cd exposure, and the content was much higher in the roots than the leaves (Fig. 2), an indicative of drastic phytochelatin formation for Cd chelation. The content of Cys also increased in both leaves and roots, and the content was higher in leaves than in roots (Fig. 3). Total glutathione content increased significantly only in leaves (Fig. 4). In roots, no significant changes of total glutathione might be induced by the rapid synthesis of thiols including PC. However, an increase in the ratio of GSSG to GSH was invariably observed in both leaves and roots, indicating more oxidation of the glutathione pool under Cd stress (Fig. 5). The large amounts of thiols synthesized in tomato seedlings exposed to Cd have also been observed in a number of other plant species (Scheller et al. 1987, Vogeli-Lange and Wagner 1996).

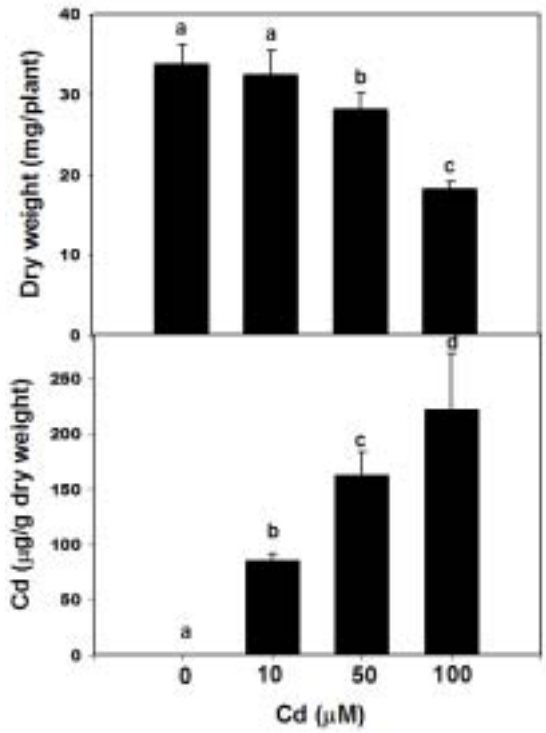


Fig. 1. Dry weight and Cd content in tomato seedlings exposed to Cd for 9 days. Values are mean+SE of five independent replicates. Values with the same letters are not significantly different at the 0.05 levels according to Duncan's multiple range tests.

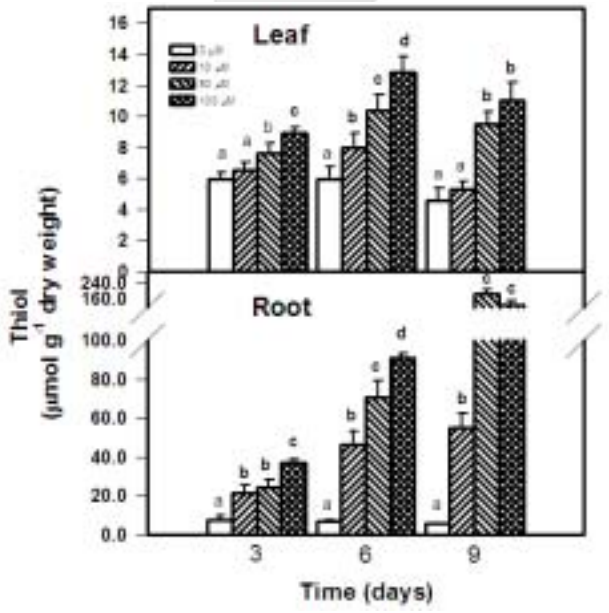


Fig. 2. Concentration of total non-protein thiols in leaves and roots of tomato seedlings exposed to Cd for 9 days. Values are mean+SE of five independent replicates. Values with the same letters are not significantly different at the 0.05 levels according to Duncan's multiple range tests.

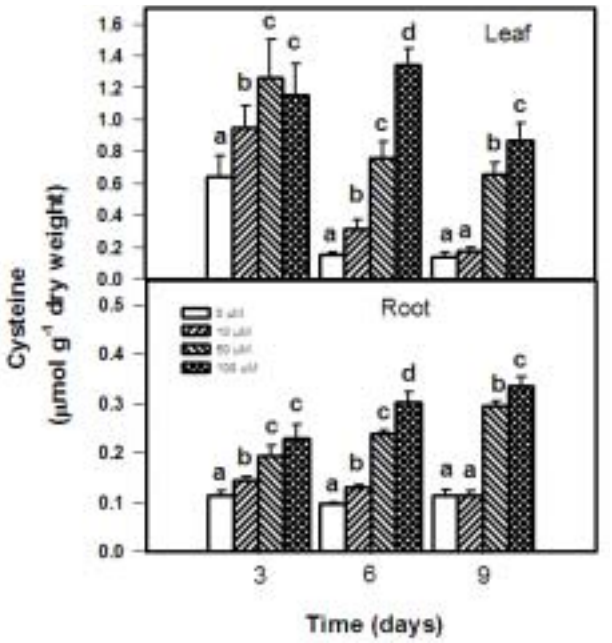


Fig. 3. Concentration of total glutathione in leaves and roots of tomato seedlings exposed to Cd for 9 days. Values are mean+SE of five independent replicates. Values with the same letters are not significantly different at the 0.05 levels according to Duncan's multiple range tests.

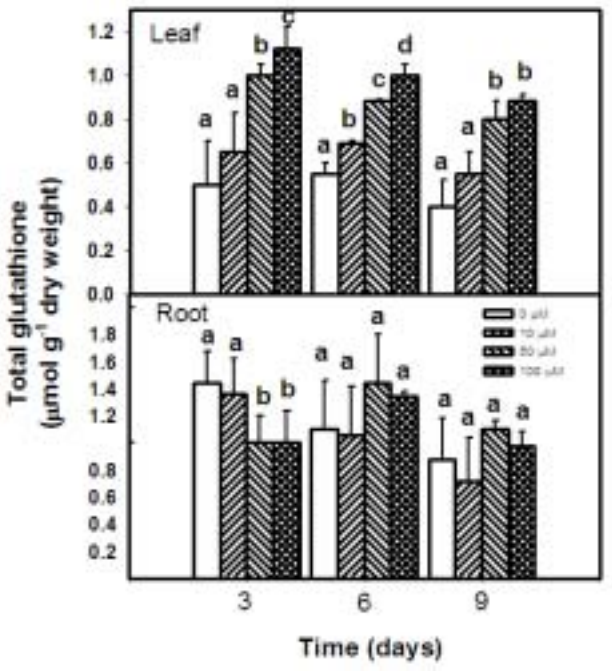


Fig. 4. Concentration of cysteine in leaves and roots of tomato seedlings exposed to Cd for 9 days. Values are mean+SE of five independent replicates. Values with the same letters are not significantly different at the 0.05 levels according to Duncan's multiple range tests.

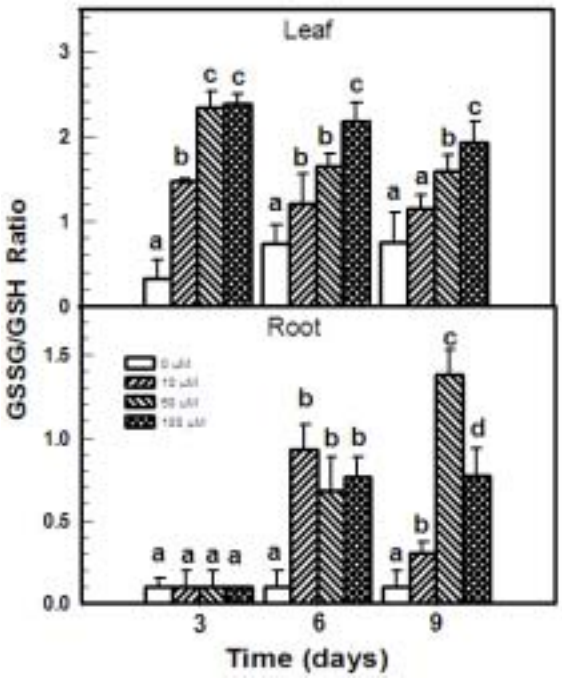


Fig. 5. GSSG/GSH ratio in leaves and roots of tomato seedlings exposed to Cd for 9 days. Values are mean+SE of five independent replicates. Values with the same letters are not significantly different at the 0.05 levels according to Duncan's multiple range tests.

The cellular concentration of GSH declines as thiols are synthesized in response to the addition of Cd to the medium (Figs. 2 and 3 in this study, Jackson et al. 1992, Ruegsegger et al. 1992). Therefore, an increase in total glutathione observed in our experiment might be due to the increase of GSSG. The increase of GSSG could be a result of the increased activity of peroxide scavenging, and Cd-induced PC synthesis might result in the lowered GSH level in cytosol, where PCs are made and have a high affinity for binding with heavy metals particularly Cd and Cu (Grill et al. 1985, Ha et al. 1999).

Glutathione plays a crucial role in controlling and maintaining the intracellular redox state (Noctor and Foyer 1998). The size and redox state of glutathione pool changes in response to various environmental factors including temperature (Wise and Naylor 1987), light (Noctor et al. 1998) and heavy metals (Scheller et al. 1987, Ruegsegger and Brunold 1992, Schneider and Bergmann 1995). GSH synthesis can occur in the chloroplast and the cytosol (Noctor et al. 1998b) and respond to the availability of amino acid substrates, particularly Cys (Buwalda et al. 1990, Strohm et al. 1995, Noctor et al. 1996). Therefore, the increased levels of Cys in respose to Cd exposure (Xiang et al. 2001, Fig. 3) might be due to GSH depletion.

A shift in the glutathione redox couple to a more oxidized state and GSH depletion by metal exposure might be related to oxidative stress and metal toxicity. Copper tolerance of *Silene vulgaris* was related to their ability to prevent GSH depletion more than to protective functions of PCs (De Vos et al. 1992) and Indian mustard plants having a 1.5- to 2.5-fold increase in GSH showed some increase in metal resistance (Zhu et al. 1999). Since $H_2O_2$ level might also be increased by the depletion of GSH and the subsequent inefficient operation of GSH-ascorbate cycle-- a major $H_2O_2$ scavenging system-- GSH was assumed to be important in metal tolerance, functioning as an antioxidant against free radicals and hydrogen peroxide (Alscher 1989).

The nucleophilic nature of the thiol group is also important in the formation of mercaptide bonds with metals and for reacting with select electrophiles (Xiang et al. 2001).

ASA is widely distributed in both animals and plants, and oxidized DASA occurs in biological materials in relatively low concentrations. DASA is also formed in a redox system in the presence of ascorbic acid. As a result of Cd exposure, the ascorbic acid pool in leaves increased initially but remained unchanged with longer Cd exposure compared to controls (Fig. 6). However, in the same conditions, it increased initially, but decreased progressively later in the leaves. As a result of these changes, the DASA to ASA ratio increased initially but decreased later with Cd exposure.

Thiols also possess strong antioxidative properties and they are consequently able to counteract oxidative stress (Pichrner et al. 1993). GSH also acts as an electron acceptor and donor for numerous biological reactions (Noctor and Foyer 1998a), and is an important component of the cell's scavenging system for ROS (Halliwell 1986, Kunert and Foyer 1993). Most of the metabolic

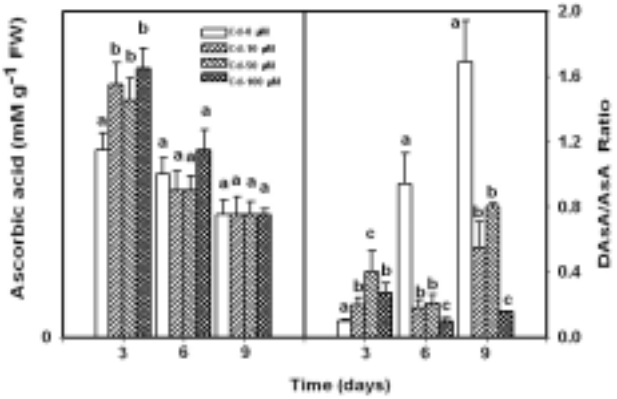


Fig. 6. Concentration of ascorbic acid and DASA/ASA ratio in leaves and roots of tomato seedlings exposed to Cd for 9 days. Values are mean+SE of five independent replicates. Values with the same letters are not significantly different at the 0.05 levels according to Duncan's multiple range tests.

functions of GSH involve the oxidation of reduced GSH to oxidized glutathione disulfide (GSSG). The GSH-ascorbate cycle (Foyer et al. 1994) is essential in removing $H_2O_2$ (Asada 1999), and the activities of enzymes involved in the cycle depend on the availability of ASA and GSH. In response to various environmental factors including temperature (Wise and Naylor 1987), light (Noctor et al. 1996) and heavy metals (Scheller et al. 1987, Ruegsegger and Brunold 1992, Schneider and Bergmann 1995), the size or redox state of the GSH pool changes. GSH might be required for both PC synthesis and ROS scavenging and might be a key element for surviving particularly in plants exposed to heavy metals, and the decision of plants to use GSH for PC synthesis to detoxify metals or $H_2O_2$ scavenging to prevent oxidative stress might be a characteristic response to metal exposure. Therefore, our results might explain the Cd-induced ROS formation and oxidative stress. In fact, heavy metals are known to be involved in many ways in the production of ROS including $H_2O_2$ (Halliwell and Gutterrifge 1984), and it is conceivable to suppose that a decrease of enzymic and non-enzymic free radical scavengers caused by heavy metals (De Vos et al. 1993) may contribute to the shift in the balance of free-radical metabolism towards ROS accumulation. The enhanced production of $H_2O_2$ by Cd exposure (Cho 2004) could also contribute to the enhanced lipid peroxidation. Further interaction of $H_2O_2$ and $O^{\cdot}_2$ in the presence of metal ions or metal chelates may produce highly reactive hydroxyl radicals ($^{\cdot}OH$). Since Cd exposure increased TBA-RS, lipid peroxidation products (Cho 2004), it was clear that the decreased growth of these seedlings during Cd exposure could be due to the increased levels of oxidative damage.

The increases in glutathione reductase (GR) activity in both leaves and roots (Cho 2004) appeared to be due to active thiol synthesis and the increased GSSG levels. GR in plastid and cytosol is a highly specific enzyme that utilizes NADPH to reduce GSSG to 2 molecules of GSH (Schaedle 1977) and can maintain a high ratio of GSH/GSSG and by this means plays an important role in the regulation of cell metabolism (Williams 1976). Therefore, overexpression of GR in the plastids showed enhanced Cd tolerance in terms of chlorosis and chlorophyll fluorescence parameters, and the lower Cd stress in the transgenic plants could be the result of increased GSH synthesis and subsequent increase of thiols for metal binding in roots and reduced Cd translocation in shoots (Pilon-Smits et al. 2000). However, the higher GSSG : GSH ratios observed might indicate that GR activity in seedlings exposed to Cd was not sufficient to provide GSH for ROS quenching and thiol synthesis. Meanwhile, the increase of GSSG might be a result of the increased activity of peroxide scavenging and active PC synthesis. The lowered GSH-dependent $H_2O_2$ quenching pathway due to GSH depletion might lead to increased GSSG accumulation and, perhaps, lower ASA utilization. This reduced demand for ASA could contribute to the decreased DASA/ASA ratio particularly after day 6. Therefore, depletion of GSH due to synthesis of PC in the presence of heavy metals may result in an increase in oxidative stress (De Vos et al. 1993). Accumulation of GSSG might also be a result of the increased GPX-dependent peroxide scavenging (Roxas et al. 2000). Glutathione peroxidase is capable of rapidly scavenging products of lipid peroxidation generated as a result of enhanced ROS production (Bartling et al. 1993) as well as endogenous reactive products of cellular metabolism (Rushmore and Pickett 1993).

Based on our results, we conclude that the rapid physiological damage as inferred from the reduction of seedling growth could result from oxidative stress. The oxidative stress could be a result of the GSH depletion due to synthesis of thiols such as PC and subsequent inefficient quenching of $H_2O_2$ by the altered GSH-ascorbate cycle.

## ACKNOWLEDGEMENT

This work was supported by a grant from Changwon National University made in the 2005 academic year.

## LITERATURE CITED

Alscher RG. 1989. Biosynthesis and antioxidant function of glutathione in plants. Physiol Plantarum 77: 457-464.

Asada K. 1992. Ascorbate peroxidase – A hydrogen peroxide scavenging enzyme in plants. Physiol Plantarum 85: 235-241.

Barcelo J, Poschenrieder C. 1990. Plant water relations as affected by heavy metal stress: A review. J Plant Nutr 13: 1-37.

Buwalda F, Stulen I, De Kok LJ, Kuiper PJC. 1990. Cysteine, $\gamma$-glutamylcysteine and glutathione contents of spinach leaves as affected by darkness and application of excess sulfur. II. Glutathione accumulation in detached leaves exposed to $H_2S$ in the absence of light is stimulated by the supply of glycine to the petiole. Physiol Plantarum 80: 196-204.

Cho UH, Park JO. 2000. Mercury-induced oxidative stress in tomato seedlings. Plant Science 156: 1-9.

Cho UH. 2004. Cadmium-induced phytotoxicity in tomato seedlings due to the accumulation of $H_2O_2$ that results from the reduced activities of $H_2O_2$ detoxifying enzymes. Korean J Ecol 27: 107-114.

Cho UH, Seo NH. 2005. Oxidative stress in *Arabidopsis thaliana* exposed to cadmium is due to hydrogen peroxide accumulation. Plant Science 168: 113-120.

Chaoui A, Mazhoudi S, Ghorbal MH, El Ferjani E. 1997. Candmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.). Plant Science 127: 139-147.

De Vos CHR, Schat H, De Waal MAM, Vooijs R, Ernst WHO. 1991. Increased resistance to copper-induced damage of the root cell plasmalemma in copper-tolerant *Silene cucubalus*. Physiol Plant 82: 523-528.

De Vos CHR, Vonk M, Vooijs R, Schat H. 1992. Glutathione depletion due to copper-induced phytochelatin synthesis causes oxidative stress in *Sulene cucubalus*. Plant Physiol 98: 853-858.

De Vos CHR, Ten Boukum WM, Vooijs R, Schat H, De Kok LJ. 1993. Effect of copper on fatty acid composition and peroxidation of lipids in the roots of copper-tolerant and ‐sensitive *Silene cucubalus*. Plant Physiol Biochem 31: 151-158.

Delhaize E, Jackson PJ, Lujan LD, Robisnson NJ. 1989. Poly ( $\gamma$-glutamylcysteinyl) glycine synthesis in *Datura innoxia* and binding with cadmium. Plant Physiol 89: 700-706.

Dixit V, Pandey V, Shyam R. 2001. Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L. cv. Azad). J Exp Bot 52: 1101-1109.

Ebbs S, Lau I, Ahner B, Kochian LV. 2002. Phytochelatin synthesis is not responsible for Cd tolerance in the Zn/Cd hyperaccumulatior *Thlapsi* (J&C. Presl). Planta 214: 635-640.

Ernst WHO, Nelissen HJM, Ten Bookum WM. 2000. Combination toxicology of metal-enriched soils: physiological responses of a Zn- and Cd-resistant ecotype of *Silene vulgaris* on polymetallic sols. Env Exp Bot 43: 55-71.

Fadzilla NM, Finch RP, Burdon RH. 1997. Salinity, oxidative stress and antioxidant responses in shoot cultures of rice. J Exp Bot 48: 325-331.

Foyer CH, Lelandais M, Kunert KJ. 1994. Photooxidative stress in plants. Physiol Plant 92: 696-717.

Gaitonde MK. 1967. A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. Biochem J 104: 627-633.

Grill E, Winnacker E-L, Zenk MH. 1985. Phytochelatins: the principal heavy-metal complexing peptites of higher plants. Science 230: 674-676.

Ha SB, Smith PAP, Howden R, Dietrich WM, Bugg S, O'Connell MJ, Goldsbrough PB, Cobbett CS. 1999. Phytochelatin synthase genes from Arabidopsis and the yeast *Schizosaccharomyces pombe*. Plant Cell 11: 1153-1163.

Halliwell B, Gutteridge JMC. 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem J 219: 1-14.

Hegedus A, Erdei S, Horvath G. 2001. Comparative studies of $H_2O_2$ detoxifying enzymes in green and greening barley seedlings under cadmium stress. Plant Science 160: 1085-1093.

Hoagland DR, Arnon DI. 1938. The water culture method for growing plants without soil. Cal Agri Exp Station Circular 347: 1-39.

Jackson PJ, Unketer CJ, Doolen IA, Katt K, Robinson NJ. 1987. Poly ( $\gamma$-glutamylcysteinyl) glycine: its role in cadmium reistance in plant cells. Proc Natl Acad Sci USA 84: 6619-6623.

Kessler A, Brand MD. 1995. The mechanism of the stimulation of state 4 respirartion by cadmium in potato tuber (*Solanum tuberosum*) mitochondria. Plant Physiol Biochem 33: 519-528.

Kupper H, Mijovilovich A, Meyer-Klaucke W, Kroneck PMH. 2004. Tissue- and age-dependent differences in the complexation of cadmium and zinc in the cadmium/zinc hyperaccumulator *Thlaspi caerulescens* (Ganges Ecotype) revealed by X-ray absorption spectroscopy. Plant Physiol 134: 748-757.

Law MY, Charles SA, Halliwell B. 1983. Glutathione and ascorbic acid in spinach (*Spinacia oleracea*) chloroplasts. Biochem J 210: 899-903.

Lee S, Moon JS, Ko TS, Petros D, Goldsbrough PB, Korban SS. 2003. Overexpression of Arabidopsis phytochelatin synthase paradoxically leads to hypersensitivity to cadmium stress. Plant Physiol 131: 1-8.

Mehra RK, Tripathi RD. 2000. Phytochelatins and metal tolerance. In : Environmental Pollution and Plant Responses (Agrawal SB, Agrawal M, eds). CRC Press, Boca Raton. pp 367-382.

Noctor G, Strohm M, Jouanin L, Kunert KJ, Foyer CH, Rennenberg H. 1996. Synthesis of glutathione in leaves of transgenic poplar (*Populus tremula* × *P. alba*) overexpressing $\gamma$-glutamylcysteine synthetase. Plant Physiol 112: 1071-1078.

Noctor G, Foyer CH. 1998. Ascorbate and glutathione: Keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol 49: 249-279.

Noctor G, Arisi ACM, Jouanin L, Kunert KJ, Rennenberg H, Foyer CH. 1998a. Glutathione: Biosynthesis, metabolism and relationship to stress tolerance explored in transformed plants. J Exp Bot 49: 623-647.

Noctor G, Arisi ACM, Jouanin L, Foyer CH. 1998b. Manipulation of glutathione and amino acid biosynthesis in the chloroplast. Plant Physiol 118: 362-372.

Ouariti O, Boussama N, Zarrouk M, Cherif A, Ghorbal MH. 1997. Cadmium- and copper-induced changes in tomato membrane lipids. Phytochemistry 45: 1343-1350.

Pilon-Smits EAH, Zhu Y, Sears T, Terry N. 2000. Overexpression of glutathione reductase in *Brassica juncea*: effects on cadmium accumulation and tolerance. Physiol Plantarum 110: 455-460.

Rauser WE. 1987. Changes in glutathione content of maize seedlings exposed to cadmium. Plant Science 51: 171-175.

Rauser WE. 1995. Phytochelatins and related peptides: Structure, biosynthesis, and function. Plant Physiol 109: 1141-1149.

Ruegsegger A, Brunold C. 1992. Effect of cadmium on $\gamma$-glutamylcysteine synthesis in maize seedlings. Plant Physiol 99: 428-433.

Salt DE, Thurman DA, Tomsett AB, Sewell AK. 1989. Copper phytochelatins of *Mimulus guttatus*. Proc R Soc Lond 236: 79-89.

Sanita di Toppi LA, Gabbrielli R. 1999. Responses to cadmium in higher plants. Environ Exp Bot 41: 105-130.

Schaedle M. 1977. Chloroplast glutathione reductase. Plant Physiol 59: 1011-1012.

Schat H, Llugany M, Vooijs R, Hartley-Whitaker J, Bleeker P. 2002. The role of phytovhelatins in constitutive and adaptative heavy metal tolerances in hyperaccumulator and non-hyperaccumulator metallophytes. J Exp Bot 53: 2381-2392.

Scheller HV, Huang B, Hatch E, Goldsbrough PB. 1987. Phytochelatin synthesis and glutathione levels in response to heavy metals in tomato cells. Plant Physiol 85: 1031-1035.

Schneider S, Bergmann L. 1995. Regulation of glutathione synthesis in suspension cultures of parsley and tobacco. Bot Acta 108: 34-40.

Schutzendubel A, Schwanz P, Teichmann T, Gross K. 2001. Cadmium-induced changes in antioxodative systems, hydrogen pero-

xide content, and differentiation in Scots Pine roots. Plant Physiol 127: 887-898.

Siedlecka A, Baszynski T. 1993. Inhibition of electron flow around photosystem I in chloroplasts of Cd-treated maize plants is due to Cd-induced iron deficiency. Physiol Plantarum 87: 199-202.

Steffens JC, Hunt DF, Williams BG. 1986. Accumulation of non-protein metal-binding polypeptides ( $\gamma$ -glutamyl-cysteinyl)$_n$- glycine in selected cadmium-resistant tomato cells. J Biol Chem 261: 13879-13882.

Tukendorf A, Rauser WE. 1990. Changes in glutathione and phytochelatins in roots of maize seedlings exposed to cadmium. Plant Science 70: 155-166.

Verkleij JAC, Bast-Cramer WB, Levering H. 1985. Effects of heavy metal stress on the genetic structure of populations of *Silene cucubalus*. In: Structure and Functioning of Plant Populations (Haek J, Woldendorp JW, eds). Noord-Holland, Amsterdam, pp 355-365.

Vogeli-Lange R, Wagner GW (1996) Relationship between cadmium, glutathione and cadmium-binding peptides (phytochelatins) in leaves of intact tobacco seedlings. Plant Science 114: 11-18.

Weckx JEJ, Clijsters HMM. 1997. Zn phytotoxicity induces oxidative stress in primary leaves of *Phaseolus vulgaris*. Plant Physiol Biochem 35: 405-410.

Williams CH. 1976. Flavin containing enzymes. In: The enzymes, Vol XIII (Boer PD, ed). Academic Press, New York. pp 89-173.

Wise RR, Naylor AW. 1987. Chilling-enhanced photooxidation: evidence for the role of singlet oxygen and endogenous antioxidants. Plant Physiol 83: 278-282.

Xiang C, Werner BL, Christensen EM, Oliver DJ. 2001. The biological functions of glutathione revisited in Arabidopsis transgenic plants with altered glutathione levels. Plant Physiol 126: 564-574.

Zhu YL, Pilon-Smits EAH, Jouanin L, Terry N. 1999. Overexpression of glutathione synthetase in Indian mustard enhances cadmium accumulation and tolerance. Plant Physiol 119: 73-79.

*(Received February 1, 2006; Accepted February 21, 2006)*