KJPR Korean J.Plant.Res. 10(3) 235~240(1997)

Effect of Metals on Anti-Oxidase Activity and Isozyme patterns in *Brassica juncea*

Hyung Jin Jeong[1], In-Jung Lee[2], Mi Hyang Sung[1]
[1]Natural Science College, Andong National University, Andong 760-749, Korea
[2]College of Agriculture, Kyungpook Nat' l. Univ. Taegu 702-701, Korea

ABSTRACT

To study the effects of metal ions on the activity of anti-oxidase enzymes, the activity of superoxide dismutase (SOD) and peroxidase (POD) and isozyme patterns of *Brassica juncea* have been studied after treating with Cd, Cu, Zn, and Al. The activity of SOD after treating with metal ions was higher than that of untreated control. SOD activity in leaves increased by treatment of 50 ppm of Zn and 500 ppm of Al. POD in stems gave highest activity after treating with 500 ppm of Cu. When the activity was compared by plant parts, lowest POD activity was observed in leaves in which protein content was higher than other tissues. When the activity was expressed as percentage of control, SOD activity was increased after treating with metal ions. SOD activity in leaves and roots of metal treated plant was significantly increased under the metal ions stress conditions. In the roots of 50 ppm of Zn treated plant, SOD activity was extremly high. POD activity was inhibited with Cd and Zn treatment in all parts of the plant. However, in leaves and stems, there was marked increase in activity after treating with Cu. The patterns of SOD isozyme after metal treatment show that two bands were stained in all metal ion treated and that no new band appeared. POD isozyme band intensity resulting from the treatment of metal ions was in order of roots 〉stems 〉 leaves, but there was no significant difference.

Key words: Superoxide dismutase, Peroxidase, *Brassica juncea*

INTRODUCTION

Oxidative stress under the environmental adversity and polution has received much attention since activated oxygen species such as superoxide radical ($O2^{-}\cdot$), singlet oxygen ($O\frac{1}{2}$), hydrogen peroxide (H_2O_2) and hydroxy radical (OH ·) have been found to be produced during the stress conditions (Kang and Shim, 1995; Shim and Kang, 1993). All of these activated oxygen species are highly reactive and cytotoxic in all living organisms. These oxygen species can react with unsaturated fatty acids in plasma membrane or intracellular organelles and cause peroxidation of essential membrane lipids (Cadenas, 1989; Charles and Halliwell, 1980; Bowler et al., 1992). Intracellular membrane damage cause pigment breakdown and loss of carbon-fixing ability in chloroplasts. Peroxidation damage of the plasma membrane leads to leakage of cellular contents, rapid desiccation, and cell death. All living organism, especially plants due to their stationary lifestyle under constantly changing environments, developed numerous defense mechanisms to protect themselves against oxidative stress. These defenses in plants involve both enzymic and non-enzymic mechanisms. The enzymic antioxidant defenses include superoxide dismutase (SOD) that scavange the superoxide anion, and peroxidase (POD) catalase (CAT) which remove H_2O_2 very efficiently. In addition, antioxidants such as ascorbic acid, α-tocopherol, and glutathione presented high levels in plants also contribute to the protection from the oxidative stress by scavenging reactive oxygen species (Mehdy, 1994; Salin, 1987; Scandalios, 1983).

SOD is an enzyme which converts the highly reactive and hence destructive superoxide radical to hydrogen peroxide ($2O_2^{-} + 2H^{+} \rightarrow H_2O_2 + O_2$)(Charles and Halliwell,

1980). SOD presents in all living organism including plants. Hydrogen peroxide formed by the action of SOD can be detoxified into the water and oxygen molecules by the function of CAT ($2H_2O_2 \rightarrow 2H_2O + O_2$). Antioxidants are valuable as a roles to cure and prevent various diseases, food additives, senescence inhibition, cholesterol depression, and skin beauty (cosmatics)(Henning and Nielsen, 1987; Klibanov et al., 1980; Park et al., 1989).

In plants, activity of SOD and POD was increased not only by the biological stresses such as viruses, fungi, and other pathogen attack, but also by the many damaging environmental stresses such as high or low temperature, air pollutants, xenobiotics, and drought (Bowles, 1990; Endress et al., 1980; Miller and Kelley, 1989). Although, anti-oxidant enzymes activities in plants under the stress conditions mentioned above have been well established, their activity under the metal toxicity was not fully tested. The purpose of this research was to determine effect of metals on anti-oxidase activity and isozyme patterns in *Brassica juncea*.

MATERIALS AND METHODS

Brassica juncea seeds were germinated and grown in a growth chamber under a 16 h photoperiod with 24~28 ℃ for 30 days. Thirty days old plants (seedlings) were transplanted into the pots (10 cm in diameter, 6.5 cm in width) filled with 250 *ml* soil mix. As a metals, $CdCl_2 \cdot H_2O$, $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, and Al_2Cl_3 were used in this experiment. Three concentrations (50, 500, and 5,000 ppm) of each metal ions were applied as a soil drench in 50 ml of each solution per pot at 7 days after transplanting (DAT). All treatments were carried out with completely randomized 6 replications. For the enzyme activity assay, samples were taken 15 days after treatments.

Brassica juncea grown in the metal ions treated soil for 15 days was cut and devided by the plant parts. Five hundred mg of each part was ground with a mortar and pestle in 1.5 *ml* of 0.05 M phosphate buffer. The homogenate was centrifuged at 14000 rpm for 20 min at 4 ℃ and the supernatant was used to determine the activity of SOD and POD.

SOD was assayed with xanthine oxidase (XOD) and cytochrome c according to McCord and Fridovich (1969) with slight modification. The reaction was performed at 25℃ in a total volume of 1 ml containing 0.05 M phosphate buffer (10mM xanthine, 10mM cytochrome c, 0.1mM EDTA, pH 7.8), and crude extracts (about 10μl). The reaction was started by the addition of 5.5 μl XOD which was diluted twenty five times with 0.05 M potassium phosphate buffer (pH 7.8) containing 10^{-4} M EDTA. One unit of SOD was defined as the absorbance change which was required to inhibit the reduction rate of SOD by 50% at 550 nm for 2 min.

Peroxidase activity was assayed with pyrogallol as substrate. The reaction was performed in a total volume of 3 *ml* cuvette contained 2.9 *ml* of assay buffer and 100 μl of crude enzyme extract. POD activity was expressed as the absorbance change at 420 nm for 20 second.

Isoforms of SOD was separated by the method of Beauchamp and Fridovich (1971) with slight modification. Crude enzyme extracts adjusted to the same protein contents were separated by electrophoresis on 13% polyacrylamide gel. Electrophoresis was carried out at 215 V for 40 min and maintained 4 ℃ throughout the duration of experiment. Following electrophoresis, gel was stained for SOD activity. After incubation in the dark for 30 min at 25 ℃ water bath in the solution containing 50 mM KH_2PO_4, 0.1 mM EDTA, 0.2% TEMED, 0.026 mM riboflavin, and 0.25 mM nitroblue tetrazolium, gel was rinsed and placed on a glass sheet. Light was illuminated until the SOD isoenzymes were visualized as a clear bands on the gel against a blue/purple background (Chung et al., 1995).

For the separation of POD isoenzymes, electrophoresis was carried out on 12.5% polyacrylamide gel at 15 mA for 40 min followed by 25 mA for 90 min. In order to detect the POD activity, gel was incubated in 1:1 mixture of benzidine solution (1 g benzidine, 9 *ml* acetic acid, and 36 *ml* distilled water) : 3% hydrogen peroxide. Throughout the electrophoresis, the system was maintained 4℃. Protein content was determined by

Table 1. Effect of metals on superoxide dismutase and peroxidase activity in *Brassica juncea*.

Plant species	Metals	Conc. (ppm)	SOD activity			POD activity		
			Leaf	Stem	Root	Leaf	Stem	Root
			activity(U/mg protein)			activity(U/mg protein)		
	control		30,635	77,469	14,706	280,	1,186	2,546
Brassica juncea	Cd	50	225,571	95,069	13,605	240	1,436	2,546
		500	64,104	49,020	62,277	188	629	2,085
		5,000	32,907	104,543	77,357	177	508	1,285
	Cu	50	74,941	82,735	147,500	227	1,913	3,250
		500	161,912	118,878	71,440	1,098	9,505	1,719
		5,000	52,586	36,876	162,319	1,329	1,589	2,148
	Zn	50	707,547	164,600	394,737	193	1,105	2,092
		500	59,016	91,750	256,384	1,458	925	3,387
		5,000	165,109	51,455	47,083	181	865	1,750
	Al	50	78,683	169,391	187,250	271	497	1,588
		500	380,952	131,628	13,700	462	1,030	1,050
		5,000	72,472	77,965	33,870	229	872	2,729

the Bradford' s method (1976).

RESULTS AND DISCUSSION

The activity of SOD and POD were measured at 15 days after metal ions treatments. SOD activity in the metal ions treated plants was higher than that of untreated control (Table 1). Although there is no consistent trend in SOD activity by the metal treatment when activity was compared by the plant parts, SOD activity was high in the 50 ppm of Cd or Zn and 500 ppm of Cu or Al treated plants. In the leaves of 50 ppm of Zn and 500 ppm of Al treated plant, SOD activity was extremly high (707,547 and 380,952 unit/mg protein, respectively). The dose-response curve for the metal ions showed that Zn treatment most strongly promoted plant SOD activity throughout the concentrations tested, while Cd did not promote SOD activity. However, since uptake, translocation, and metabolic activation could be different between these metal ions, it is very difficult to correlate the effect of individual metal on the plant anti-oxidant enzyme activity with the concentration itself of each metal.

POD activity also showed same trend as SOD. Thus, there is no consistent trend in POD activity. Nevertheless, POD activity in the stem tissues of *Brassica juncea* treated with 500ppm of Cu was relatively high (9,505 unit/mg protein). When the activity was compared by the plant parts, lowest POD activity was observed in leaf tissues in which protein content was higher than other tissues. This result was similar to those reports by Miller and Kelly (1989) and Antije et al. (1996). In those studies, protein content was highest in an extract of leaves, but protein related peroxidase activity was lower in leaves than in rooots.

When the activity was expressed as percentage of control, SOD activity was increased by metal ions treatment (Fig. 1). SOD activity in stem tissues of metal treated plant was similar to that of untreated control. However, in leaf and root tissues, activity was significantly increased under the metal ions stress conditions. Highest SOD activity (2,684%) was observed at 50 ppm of Zn treated root tissues. Generally, SOD activity was 2~5 times higher in Zn or Al treated sample than Cd or Cu treated one. High SOD activity has been linked with stress tolerance in plants. Increase in the activity has been reported during exposure of cold-acclimated spinach to low-temperature

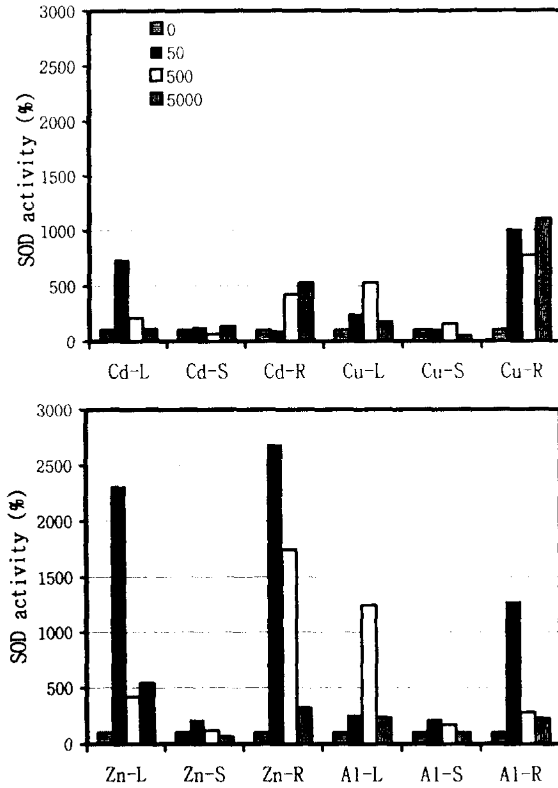

Fig 1. Superoxide dismutase activity in *Brassica juncea*.
SOD activity was expressed as percentage of control (0ppm)
L:Leaf, S:Stem, R:Root, Control:100%

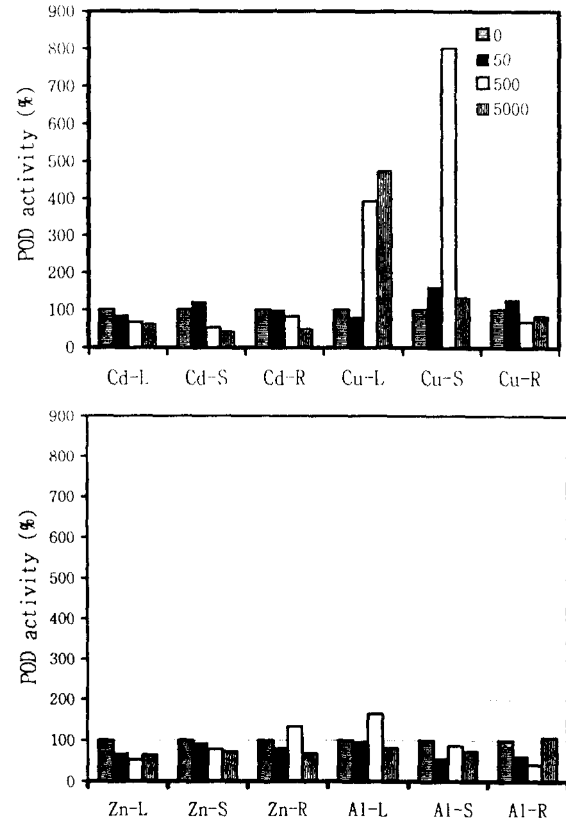

Fig 2. Peroxidase activity in *Brassica juncea*.
POD activity was expressed as percentage of control (0ppm)
L:Leaf, S:Stem, R:Root, Control:100%

and high-irradiance stress, drought conditions, and chilling. We presume that a similar kind of induction of SOD biosynthesis might be occurring in our experiments in which plants were subjected to metal ions stress conditions.

Peroxidase activity was progressively inhibited in all plant parts tested by increasing the concentrations of Cd and Zn (Fig. 2). In contrast, treatment with Cu promoted POD activity in both leaves and stems. POD activity markdly increased in both leaves and stems when 500 and 5,000 ppm of Cu was treated, and showed 391.7% and 474% as expressed percentage of control, respectively. However, this result on POD activity is contrast to the effect of Cu on sunflower POD activity where Cu inhibit POD activity in both roots and leaves, in apoplast and cell material (Garcia et al., 1996).

The patterns of SOD isozyme after metal treatment are shown in Fig. 3. SOD isozyme pattern show that two bands were stained in all metal ion treated and that no new band appeared. As the Cd concentration increased, SOD band in shoots and roots was decreased in intensity, and eventually disappeared at 5,000 ppm of Cd treatment. This result was similar to that of Sung et al. (1996). With the application of Cu, the SOD band in leaves and stems are strongly expressed. In contrast, its band in roots disappeared with Cu treatment. In case of Zn, there is no significant changes in SOD activity by the concentrations differenece. However, in roots of *Brassica juncea* treated with Zn, SOD band disappeared at Zn

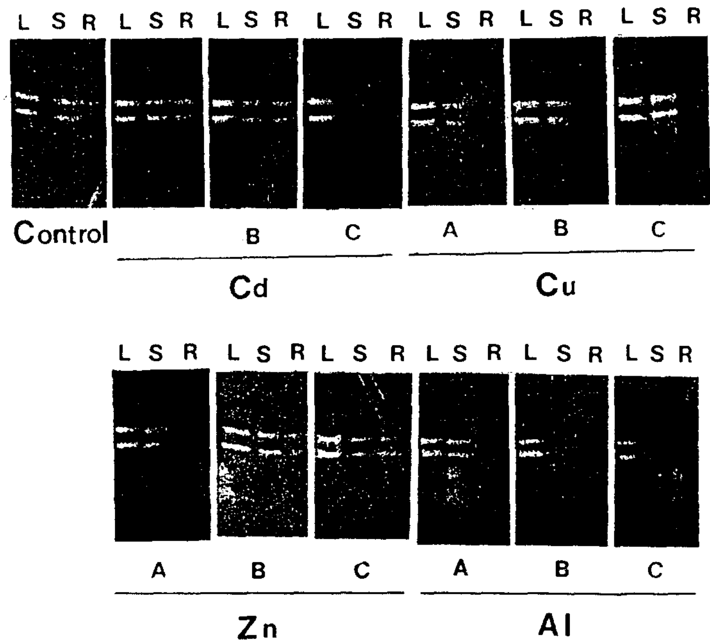

Fig 3. The patterns of SOD isozymes in *Brassica juncea*.
SOD was detected by nitro blue tetrazolium stainning after native PAGE.
L:Leaf, S:Stem, R:Root,
A:50ppm, B:500ppm, C:5,000ppm, Cont:Control

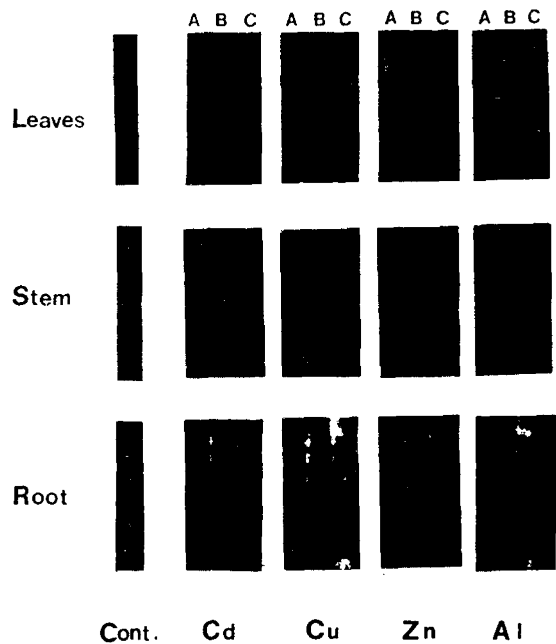

Fig 4. The patterns of POD isozymes in *Brassica juncea*.
POD was detected by nitro benzidine solution stainning after native PAGE.
A:50ppm, B:500ppm, C:5,000ppm, Cont:Control

concentration as low as 50 ppm.

POD isozyme band intensity resulting from the treatment of metal ions was in order of roots> stems> leaves (Fig. 4). POD band in leave tissues was the most faintly stained, and did not show any significant difference. This result was also similar to that of Sung at al. (1996). POD activity in roots also did not changed by metals treatment. In stem tissues, one band was appeared by 5,000 ppm of Cd treatment, but band disappeared by Cu treatment.

REFERENCES

Antije B., Ahmed A.M., Marina A., Andreas B., Karl J.D. 1996. Heavy metal induced changes in peroxidase activity in leaves, roots and cell suspension cultures of *Hordeum vulgare* L. Plant peroxidases : Biochemistry and Physiology, Obinger, C., Burner, U., Ebermann, R., Penel, C. and Greppin, H. eds. pp374-379.

Beauchamp, C. and Fridovich, I. 1971. Superoxide dismutase : Improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44:276-287.

Bowler, C., Montagu, M.V. and Inz, D. 1992. Superoxide dismutase and peroxidase activity in root tips of soybean (*Glycine max*). Physiol. Plant. 83:463-468.

Bowles, D.J. 1990. Defence-related proteins in higher plants. Ann. Rev. Biochem. 59:873-907.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72:248-254.

Cadenas, E. 1989. Biochemistry of oxygen toxicity. Ann. Rev. Biochem. 58:79.

Charles, S.A. and Halliwell, B. 1980. Effects of hydrogen peroxide on spinach (*Spinacia oleraceae*) chloroplast fructose-bisphosphate. Biochem. J. 189:373.

Chung, I.M., Yun, S.J., Kim, J.T., Gwag, J.G., Sung J.D. and Suh, H.S. 1995. Test of superoxide dismutase characteristics and antioxidant activity in perilla leaves. Kor. J. Crop Sci. 40(4):504-511

Endress, A.G., Suarez, S.J. and Taylor, O.C. 1980. Peroxidase activity in plant leaves exposed to gaseous HCl of ozone. Environ. Pollut. 22:47-58.

Garcia, A., Navarro, P. and Castillo F.J. 1996. Peroxidase and NADPH oxidase activities in leaves and roots of sunflower plants as markers of heavy metals toxicity. Plant Peroxidases : Biochemistry and Physiology. pp369-373.

Henning, D. and Nielsen, K. 1987. Peroxidase labelled monoclnal antibodies for use in enzyme-immunoassay. J. Immunoassay 8:297-308.

Kang, B.H. and Shim, S.I. 1995. Paraquat toxicity in weed species: Difference in physiological responses between tolerant and susceptible species. Kor. J. Weed Sci. 15(3):224-231.

Klibanov, A.M., Albert, B. N., Norriss, E.D., Felshin, L. M. 1980. Enzymatic removal of toxic phenols and anilines from waste waters. J. Appl. Biochem. 2:414-421.

McCord, J.M., Fridovich, I. 1969. Superoxide dismutase. An enzymic function for erythrocuprein(Hemocuprein). J. Biol. Chem. 244:6049-6055.

Mehdy, M.C. 1994. Active oxygen species in plant defense against pathogens. Plant. Physiol. 105:467-472.

Miller, A.R., Kelley, T. J. 1989. Mechanical stress stimulates peroxidase activity in cucumber fruit. Hortscience. 24 :650-652.

Park, I.S., Kho, S.O., Nam, I. 1989. Korean-radish peroxidase for enzymatic determination of glucose. J. Korean Biochem. 22:411-416.

Salin, M.L. 1987. Toxic oxygen species and protective systems of the chloroplast. Physiol. Plant. 72:681-689.

Scandalios, J.G. 1983. Oxygen stress and superoxide dismutase. Plant Physiol. 101:7-12.

Sung, M.H., Jeong, H.J., Kim, K.W. and Kwak, S.S. 1996. Effect of metals on anti-oxidase activity in *Persicaria vulgaris* Webb. et Moq. Kor. J. Weed Sci. 16(4):346-353

Shim, S.I. and Kang, B.H. 1993. Oxidative stress resulting from environmental pollutions and defence mechanism in plants. Korean J. Environ Agric 12(3):264-280