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Aluminum Stress in the Roots of Naked Barley

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Phytotoxicity of aluminum (Al) is the major limiting factor for the crops grown in acid soils rapidly inhibiting root elongation. In this study, changes in root growth, total activity and isozyme patterns of antioxidant enzymes such as peroxidase, ascorbate peroxidase, catalase and glutathione reductase by Al stress were investigated in the roots of naked barley (*Hordeum vulgare* L. cv. Kwangwhalssalbori). As Al concentration increased up to 500 M, the rooting rate and root elongation substantially decreased. Growth results suggested that this cultivar is an Al-sensitive species. Total activities of antioxidant enzymes generally increased at lower Al concentrations and then gradually decreased at higher Al concentrations. They also increased when the exposure time to Al was extended up to 48 hr. Changes in the isozyme patterns of antioxidant enzymes were investigated by *in situ* enzyme activity staining on a non-denaturing PAGE gel. They generally coincided with the changes in the total activity in parallel. Changes in the total activity of antioxidant enzymes also coincided with the changes of the root growth. Since growth reduction in the roots by Al stress could be related with the changes in the activities of antioxidant enzymes, these results suggested that Al might cause the oxidative stress in the roots of this cultivar of naked barley.

Keywords: aluminum stress, antioxidant enzyme, isozyme patterns, naked barley, root growth

Phytotoxicity of aluminum is a major limiting factor for the crops grown in acid soils. The major symptoms of Al toxicity are rapid inhibition of root growth (Fig. 1) and uptake of the nutrients that ultimately results in a loss of productivity (Kochian, 1995).

Acid soils at present comprise up to 40% of the world's arable lands. As days go by, acidification of the soils due to the fertilizer usage and acid rain by industrialization has impaired the buffering capacities of soils leading to toxic levels of Al (Sparks, 1995). There is a need to improve agricultural output on these infertile soils and also to understand the cause. Although considerable researches have been made to elucidate the mechanisms of Al toxicity and resistance in plants in recent years, there has been a lack of understanding of the fundamental process underlying plant response to Al (Kochian, 1995).

When a plant is under stress, the production of activated oxygen species (AOS) can exceed the capacity of the scavenging systems, resulting in oxidative damage (Price et al., 1994). For the protection from oxidative stress, plants possess enzymatic antioxidative defense systems in the cell organelles. Catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX) and glutathione reductase (GR) are the major constituents of the plant antioxidant enzyme system by scavenging AOS (Anderson et al., 1995; Rao et al., 1996).

The changes of antioxidant enzyme activities in other various stresses such as heavy metal (Cho and Park, 1999), herbicides (Kang et al., 1998), salt and wounding have been reported so far but the changes in antioxidant enzymes in relation to Al stress have not been reported yet. Here, we investigated whether Al changes the level of antioxidant enzymes in the naked barley roots and whether the Al induced changes in total activity of antioxidant enzymes reflect the changes in their isozyme compositions.

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MATERIALS AND METHODS

Plant Materials and Growth Conditions

Seeds of naked barley cultivar (*H. vulgare* L. cv. Kwangwhalssalbori) were obtained from Honam Crops Experiment Station, Iksan, Korea. They were surface-sterilized for 30 min by soaking in 10% chlorax solution and then washed with tap water for 30 min. The seeds were germinated on a nylon mesh which was put on a polypropylene container filled with 2 L of tap water and grown hydroponically at 27°C in the dark.

Aluminum ($AlCl_3$) Treatment

To observe the effect of different Al^{3+} concentration, intact roots of 5-day-old seedlings were soaked in a solution containing 10 mM Na-acetate (pH 4.5) and 200 μM $CaCl_2$ with $AlCl_3$ from 50 μM to 500 μM or without $AlCl_3$. After 24 h of incubation, roots were cut with a razor blade and frozen in liquid nitrogen. To check the effect of different exposure times to Al^{3+}, intact roots of 5-day-old seedlings were incubated in a solution containing 10 mM Na-acetate (pH 4.5) and 200 μM $CaCl_2$ with 100 μM $AlCl_3$ or without $AlCl_3$ for the different time intervals up to 48 h. After incubation, roots from both control and Al-treated seedlings of each cultivar were cut and frozen in liquid nitrogen.

Growth Measurement

One hundred seeds were sown and germinated on a nylon mesh which was put on a polypropylene container filled with 1 L solution containing 10 mM sodium acetate (pH 4.5), 200 μM $CaCl_2$, 50 μM $CoCl_2$, and different concentrations of $AlCl_3$ from 0 to 500 μM. The rooting rate was measured by counting the number of rooting seedlings of which roots were longer than 5 mm in 3 days after sowing as described by Voigt et al. (1998). To measure the root elongation, 10 seedlings were selected whose root length were more or less uniform in 3 days after sowing. The root length was measured in 2 days after selecting 10 seedlings as described by Goh and Lee (1999).

Extraction of Soluble Proteins

For the total activity measurement of antioxidant enzymes, 2 g of frozen roots were homogenized in 8 mL of extraction buffer containing 50 mM Na-phosphate (pH 7.0), 1 mM EDTA, 150 mM NaCl, 1% PVPP, 1 mM DTT, 2 mM ascorbate and 0.6 M aprotinin with precooled mortar and pestle. The homogenate was filtered through two layers of miracloth and the filtrate was centrifuged at 12,000*g*, 4°C for 30 min. The supernatant was used as the enzyme source for the activity assays. After quick freezing in liquid nitrogen, all extracts were immediately stored at -70°C until analysis.

For *in situ* activity measurement of the enzymes on non-denaturing PAGE, extraction method was modified for each enzyme. For catalase, since maintenance of constant electrophoretic mobility of catalase isozymes on native gels was found to require the presence of DTT, 2 g of frozen roots were ground in 1 mL of 60 mM Tris-Cl (pH 6.9) buffer containing 1 mM EDTA, 1% PVPP, 10 mM DTT, 150 mM NaCl and 0.6 μM aprotinin according to Anderson et al. (1995). For ascorbate peroxidase, 2 g of frozen root was ground in 1 mL of 0.1 μM sodium phosphate (pH 7.0) buffer containing 5 mM ascorbate, 1 mM EDTA, 1% PVPP, 150 mM NaCl and 0.6 μM aprotinin. For peroxidase whose substrate could be guaiacol, ferulic acid and diaminobenzidine, 2 g of frozen roots were homogenized in 8 mL of extraction buffer containing 50 mM Na-phosphate (pH 7.0), 1 mM EDTA, 150 mM NaCl, 1% PVPP, 1 mM DTT, 2 mM ascorbate and 0.6 μM aprotinin. After removal of the insoluble cell debries by centrifugation at 18,000*g* for 30 min, samples were concentrated using centriprep-10 at 6000*g*. All extracts were immediately stored at -70°C until being used. However, extracts for the catalase and ascorbate peroxidase assays were made fresh before electrophoresis (Kang et al., 1999).

Protein Assay and SDS-PAGE

Concentration of the soluble protein was estimated by the method of Lowry et al. (1951). Discontinuous SDS-PAGE was performed according to Laemmli (1970) using minislab gels containing 9% acrylamide. After electrophoresis, the gel was stained with 0.1% coomassie brilliant blue R-250/40% methanol/10% acetic acid for several hr. Then the gel was destained with 50% methanol/10% acetic acid for 1 h followed by fixing with 10% methanol/7% acetic acid (Laemmli, 1970).

Activity Assays of Antioxidant Enzymes

Total activity was determined by monitoring the changes of absorbance at various wavelengths with a spectrophotometer. For catalase (CAT), activity was determined by monitoring the decrease of absorbance at 240 nm due to H_2O_2 consumption for 5 min. Reaction mixture contained 100 mM potassium phosphate (pH 7.0), 10 mM H_2O_2, and enzyme extract (Rao et al., 1996). For guaiacol peroxidase (GPX), activity was determined by monitoring absorbance at 470 nm according to the method of Polle et al. (1994). Reaction mixture contained 100 mM potassium phosphate (pH 6.5), 16 mM guaiacol, 10 mM H_2O_2, and enzyme extract. For ascorbate peroxidase (APX), activity was measured according to Asada (1984) by monitoring the decrease in absorbance at 290 nm as ascorbate was oxidized. Reaction mixture contained 50 mM potassium phosphate (pH 7.0), 0.5 mM ascorbate, 0.25 mM H_2O_2, and enzyme extract. For

glutathione reductase (GR), activity was determined by measuring absorbance at 340 nm as described by Rao et al. (1996). Reaction mixture contained 100 mM potassium phosphate (pH 7.8), 2 mM EDTA, 0.2 mM NADPH, 0.5 mM GSSG (oxidized form of glutathione), and enzyme extract.

In Situ Enzyme Activity Staining on the Non-Denaturing PAGE

Non-denaturing PAGE was performed with slab gel apparatus using a discontinuous system (Kang et al., 1999). The final acrylamide monomer concentration in the 0.75 mm thick slab gels was 7.5% (w/v) for the separating gel and 4% (w/v) for the stacking gel. Gels were precooled to 4°C and maintained at this temperature during electrophoresis. In non-denaturing PAGE, the protein samples were not boiled and treated with the sample buffer containing neither SDS nor DTT. Gel was run at the constant voltage of 60 V until dye reached the separating gel, and the voltage was raised to 120 V while the separating gel was run (Park, 1999). For CAT isozymes, the gel was incubated in 3.27 mM H_2O_2 for 25 min, rinsed in distilled water and soaked in 1% ferric chloride-potassium ferricyanide(III) for 10 min after electrophoresis. One percent ferric chloride-potassium ferricyanide(III) was mixed immediately before use from equal volume of each 2% stock solutions (Anderson et al., 1995). After staining, solution was poured off and the gel was briefly rinsed and stored in distilled water. It was kept in the dark except when being observed. CAT bands were yellow on a dark greenfield and were stable for several hr (Woodbury et al., 1971). For APX isozymes, non-denaturing PAGE was performed as above except the carrier buffer with 2 mM ascorbate (Kang et al., 1999; Park, 1999). The gel was prerun for 30 min to allow ascorbate to enter the gel prior to the application of the samples. All steps described below were performed at room temperature. Subsequent to the electrophoretic separation, the gel was equilibrated with 50 mM sodium phosphate buffer (pH 7.0) with 2 mM ascorbate for 30 min; the equilibration buffer was changed every 10 min. The gels were then incubated in 50 mM sodium phosphate buffer (pH 7.0) containing 4 mM ascorbate and 2 mM H_2O_2 for 20 min. H_2O_2 was added to this solution immediately prior to incubation. The gel was subsequently washed with 50 mM sodium phosphate buffer (pH 7.0) for 1 min and submerged in a solution of 50 mM sodium phosphate buffer (pH 7.8),

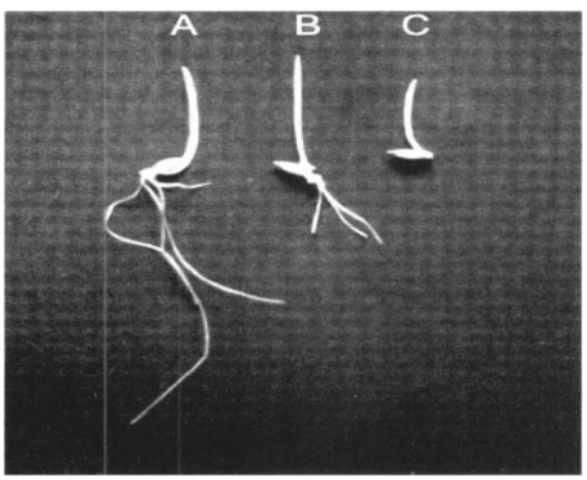

Figure 1. Inhibition of the root elongation in 4-day-old naked barley seedlings. Seedling grown in the tap water (**A**); seedling grown in the acidic solution (pH 4.5) in the absence (**B**) and presence (**C**) of 500 μM of Al. The root growth of the seedling in (**C**) was severely inhibited by Al.

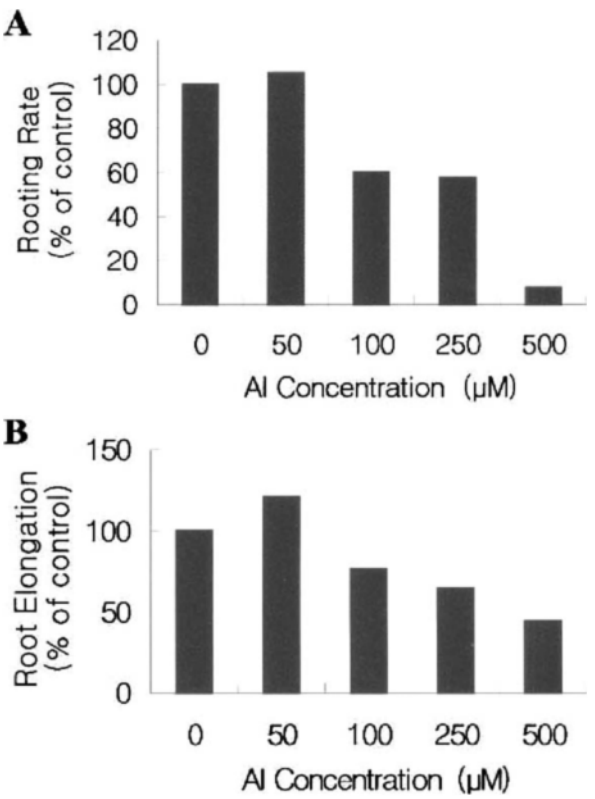

Figure 2. Reduction of rooting rate (**A**) and root elongation (**B**). As Al concentration increased, rooting rate and root elongation significantly decreased. However, slight increase of rooting rate and root elongation was observed at 50 μM of Al as compared to the control.

28 mM TEMED, and 2.45 mM NBT with gentle agitation (Mittler and Zilinskas, 1993). The APX activity was observed as a colorless band on purple-blue background (usually within 3-5 min). The reaction was allowed to continue for an additional 10 min and stopped by a brief wash with distilled water. Longer incubation with the TEMED/NBT solution resulted in a darker purple-blue background that caused some clouding of activity bands. For POX isozymes, the gel was equilibrated with 0.1 M sodium acetate buffer (pH 5.0) for 10 min and soaked in a 0.1 M sodium acetate buffer that contained 4 mM H_2O_2 and substrate (2.5 mM guaiacol, 0.5 mM ferulic acid, or 2 mM diaminobenzidine) for 10 min after electrophoresis. After staining, the gel was washed once with distilled water and photographed immediately.

RESULTS

Effects of Concentration and Exposure Time of Al on the Root Growth

Al at the various concentrations (0, 50, 100, 250 and 500 μM) induced substantial decreases in rooting rate. In addition to this, a significant inhibition by Al was also found in root elongation. However, a slight increase of rooting rate and root elongation was interestingly observed at 50 μM of Al as compared to the control (Fig. 2A and B).

Changes of Total Activity and Isozyme Pattern on Non-Denaturing Gel of Antioxidant Enzymes by Various Concentration of Al

The activity of antioxidant enzymes generally

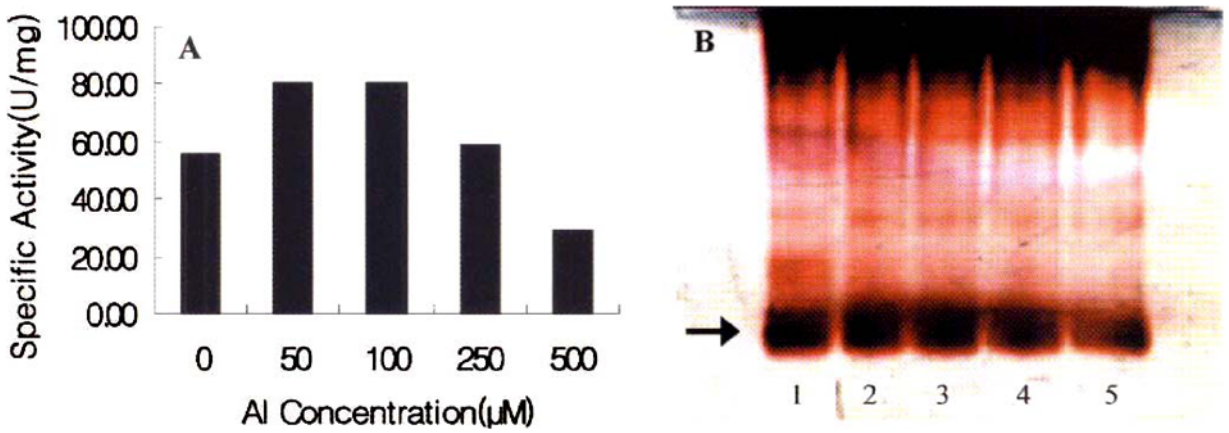

Figure 3. Changes of total activity of GPX (**A**) and GPX isozyme pattern of GPX on non-denaturing gel (**B**) at various concentrations of Al. Total GPX activity increased at 50-100 μM of Al and decreased at 500 μM of Al. GPX isozyme pattern was almost similar to the change of total activity. Lanes 1 through 5 showed GPX isozyme patterns in the presence of 0, 50, 100, 250 μM of Al, respectively.

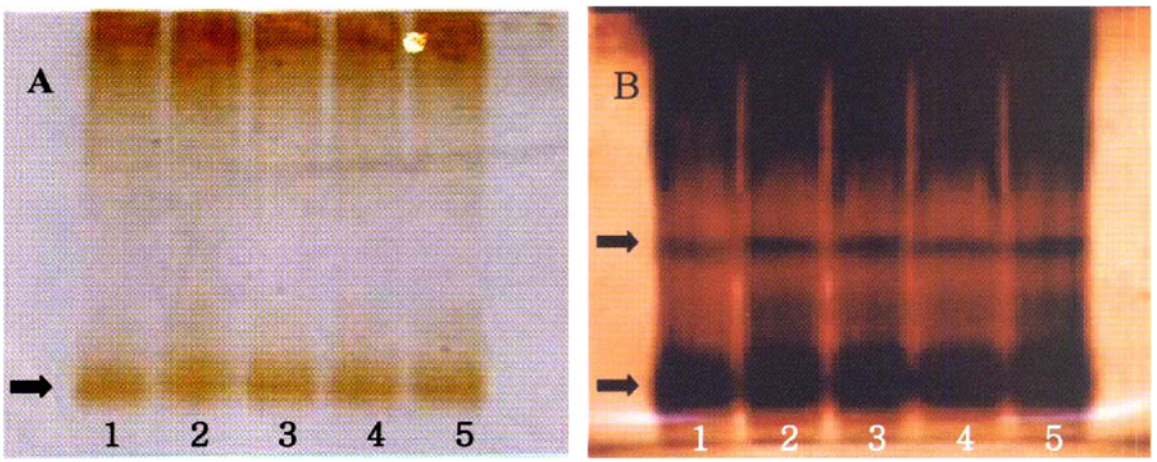

Figure 4. Changes of POX isozyme pattern on non-denaturing gel of Kwangwhalssalbori roots by various concentrations of Al. Ferulic acid (**A**) or diaminobenzidine (**B**) was used as a substrate. In both gels, a significant change was not found. Lanes 1 through 5 showed POX isozyme patterns in the presence of 0, 50, 100, 250 and 500 μM of Al, respectively.

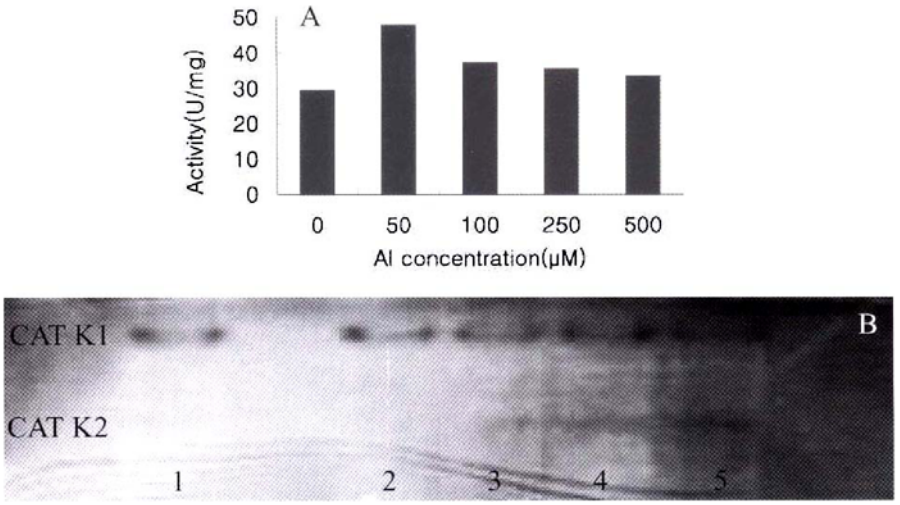

Figure 5. Changes of total activity of CAT (**A**) and isozyme pattern of CAT on non-denaturing gel (**B**) at various concentrations of Al. Total CAT activity increased at 50 μM of Al and then gradually decreased at the higher concentrations. CAT 1 activity was quite in accordance with the change of total activity. CAT 2 activity gradually increased. Lanes 1 through 5 showed CAT isozyme patterns in the presence of 0, 50, 100, 250 and 500 μM of Al, respectively.

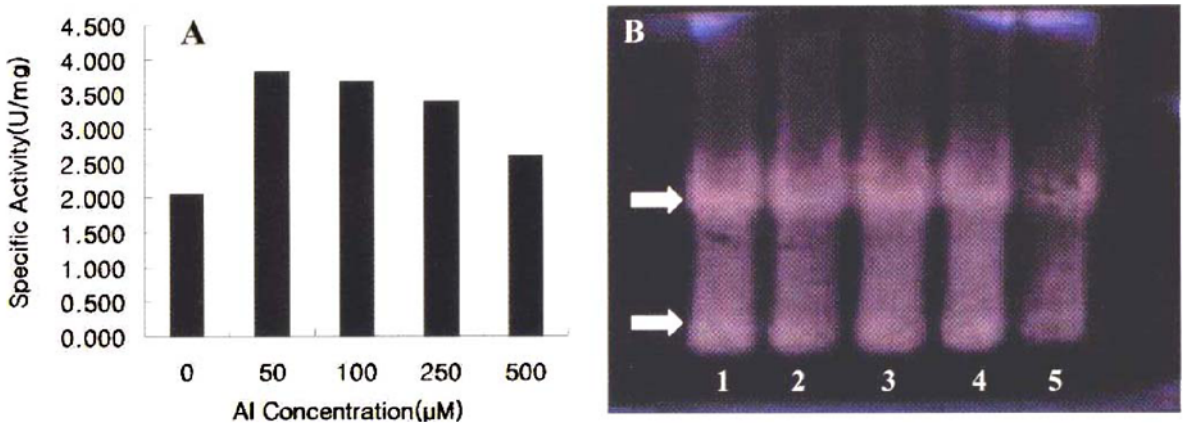

Figure 6. Changes of total activity of APX (**A**) and isozyme pattern of APX on non-denaturing gel (**B**) at various concentrations of Al. Two major isozymes showed strong activity. Lanes 1 through 5 showed APX isozyme patterns in the presence of 0, 50, 100, 250 and 500 μM of Al, respectively.

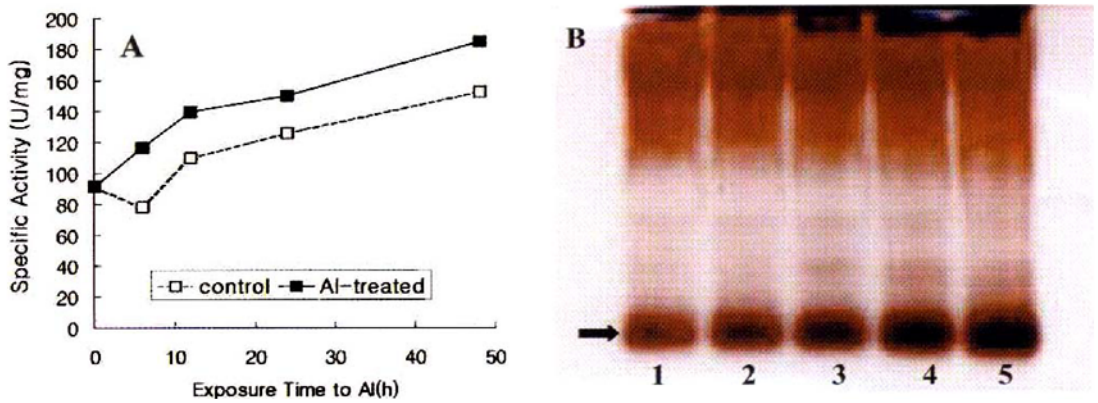

Figure 7. Changes of total activity of GPX (**A**) and isozyme pattern of GPX on non-denaturing gel (**B**) by 100 μM of Al for 48 h. Lanes 1 through 5 showed GPX isozyme pattern after Al treatment for 0, 6, 12, 24 and 48 h, respectively. The change of one isozyme (arrow mark) activity was similar to that of the total GPX activity.

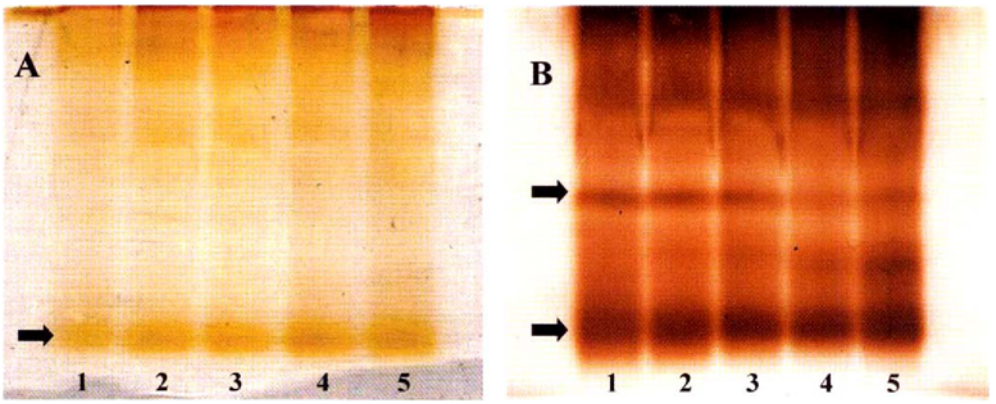

Figure 8. Changes of POX isozyme pattern on non-denaturing gel by 100 μM of Al for 48 h. Ferulic acid (**A**) or diaminobenzidine (**B**) was used as a substrate for POX. Lanes 1 through 5 showed POX isozyme patterns after Al treatment for 0, 6, 12, 24 and 48 h, respectively.

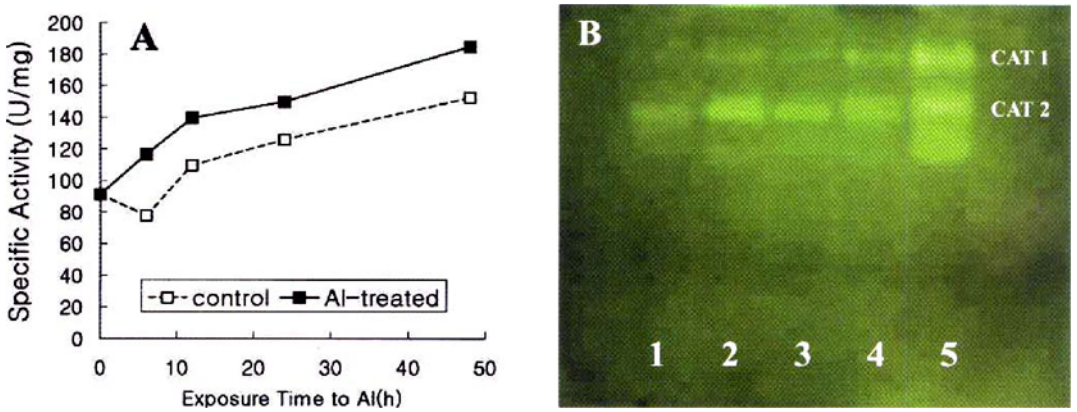

Figure 9. Changes of total activity of CAT (**A**) and isozyme pattern of CAT on non-denaturing gel (**B**) by 100 μM of Al for 48 h. Lanes 1 through 5 in (**B**) showed isozyme patterns after Al treatment for 0, 6, 12, 24 and 48 h, respectively. The changes of two isozymes, CAT1 and CAT2 mostly reflected the change of total activity.

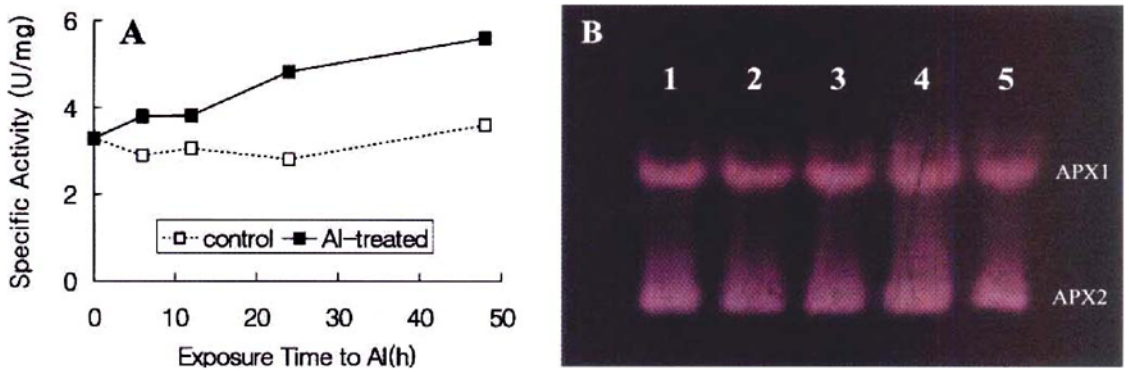

Figure 10. Changes of total activity of APX (**A**) and isozyme patterns of APX on non-denaturing gel (**B**) by 100 μM of Al for 48 h. Lanes 1 through 5 in (**B**) showed isozyme pattarns after Al treatment for 0, 6, 12, 24 and 48 h, respectively. When the exposure time to Al was prolonged, the total APX activity was increased. However, activities of APX 1 and APX 2 on the gel gradually increased up to 24 h and then decreased thereafter.

increased at the lower Al concentrations and then gradually decreased in higher Al concentrations (Figs. 3-6).

Peroxidase (POX)

The change of total activity and isozyme pattern of guaiacol peroxidase (GPX), one of the general plant

peroxidases, at the various Al concentrations was mainly examined. In addition to GPX, changes of the activities of two peroxidases, ferulic acid peroxidase (FPX) and diaminobenzidine peroxidase (DABPX), using either ferulic acid or diaminobenzidine as substrates were also examined on the non-denaturing gel.

The total GPX activity was increased at the lower Al concentration and then gradually decreased at the higher concentration (Fig. 3A). The changes of the GPX isozyme pattern were almost similar to the change of total GPX activity (Fig. 3B). However, in FPX and DABPX, a significant difference at various Al concentrations was not found (Fig. 4A and B).

Catalase (CAT)

The change of total CAT activity showed an increase at the lower Al concentrations and a decrease at the higher Al concentration, such as one of POX (Fig. 5A and B). Two CAT isozymes were detected on the non-denaturing gel. CAT 1 was quite in accordance with the change of total activity. CAT 2 gradually increased its activity on nondenaturing gel. The gel data shown in Figure 5B originally had green background and white bands but was scanned in black and white.

Ascorbate Peroxidase (APX)

Total APX activity increased up to 100 μM of Al and then gradually decreased up to 500 μM of Al (Fig. 6A). Two major bands which showed strong activity on non-denaturing gel were observed and both of them remarkably reflected the change of total APX activity (Fig. 6B).

Glutathione Reductase (GR)

Only the change of total activity was measured. The results showed a similar change to the other three antioxidant enzymes studied (data not shown).

Changes of Total Activity and Isozyme Pattern on Non-Denaturing Gel of Antioxidant Enzymes by 100 μM of Al for 48 h

As the exposure time to Al was extended up to 48 h, the total activity of antioxidant enzymes also gradually increased (Figs. 7-10). The changes of GPX and CAT isozyme pattern in the different exposure time periods to Al were almost similar to the changes of total activity (Figs. 7-9). However, APX activity on the gel gradually increased up to 24 h and then decreased thereafter (Fig. 10).

Peroxidase (POX)

When the exposure time to Al was extended, the total GPX activity gradually increased (Fig. 7A). The isozyme pattern showed a quite similar change to the total GPX activity (Fig. 7B). In the case of FPX, the same change was also observed (Fig. 8A). However, one isozyme of DABPX, which was located in the middle of the gel, showed a gradual decrease in activity (Fig. 8B).

Catalase (CAT)

As the exposure time to Al was prolonged, the total CAT activity was also gradually increased (Fig. 9A). The changes of isozyme pattern considerably reflected the changes of total activity (Fig. 9B). Especially, the increase of activity at 48 h was remarkable.

Ascorbate Peroxidase (APX)

When the exposure time to Al was prolonged, the total APX activity increased as compared to the control (Fig. 10A). Two APX isozymes, APX1 and APX2, were detected on the non-denaturing gel. Unlike the changes of total APX activities, their activities on the non-denaturing gel gradually increased up to 24 hr and then decreased thereafter (Fig. 10B).

Glutathione Reductase (GR)

Only the total activity was measured by spectrophotometer. The results showed a similar change to the previous three antioxidant enzymes (data not shown).

DISCUSSION

Effects of Al on Root Growth

The results on growth, significant reduction of rooting rate and root elongation indicated that root growth was inhibited by Al stress seriously.

Voigt et al. (1998) characterized Al effect on root growth in the acid soil by counting the number of rooting seedlings by using a soil-on-agar procedure, where a thin layer of soil is placed on top of solidified water agar, and reported that the primary factor causing a delayed rooting in the soil-on agar procedure is Al toxicity. Goh and Lee (1999) reported that Al stress inhibited the root elongation after growing the rice seedling in the presence of Al. Ishikawa and Wagatsuma (1998) proposed that most barley species can

not grow well in the acid soil. Our results supported these proposals. Even though the mechanism of Al toxicity on plant growth has not been reported yet, the results of some researchers mentioned in the examples above and our results suggest that Al stress may be a possible cause of root growth inhibition.

There is a slight increase of rooting rate and root elongation in the low Al concentration (Fig. 2A and B). Since Al concentration in the soil solution at a pH higher than 5.5 is usually much lower than 1 mg/L (~37 μM) (Ali et al., 1998), it is thought that 50 μM of Al was relatively mild stress to the naked barley. The cultivar of naked barley species used in this experiment could endure Al of low concentrations, and this kind of mild effect by Al stress has been reported in other plants. Bollard (1983) has noted the beneficial effects (growth stimulation at lower Al concentrations) on sugar beet and maize. Ali et al. (1998) also reported that regenerants of tissue cultured-*Bacopa monniera* grown on the lower concentrations (50-200 μM) of $AlCl_3$ showed a better growth compared to the control. There is no convincing evidence so far that Al is an essential mineral element even for the Al tolerant species that could grow at Al concentration as high as 1 mM (Matsumoto et al., 1976) or 6.4 mM of Al (Konishi et al., 1985).

In addition to the mild stress effects by Al, mild stress effects in relation to other environmental stresses have been reported. Mild oxidative stress induced by either chemical treatment and/or by chilling acclimation appears to be beneficial to subsequently chilled seedlings and induces a chilling tolerance (Prasad et al., 1994). The dry weight of the shoot slightly increased at lower concentrations (10 and 50 μM) of Cd during 10-day exposure to Cd (Cho and Park, 1999). Poplar trees exposed to low levels of SO_2 proved to be more resistant to subsequent damage by an exposure to high levels of SO_2 (Scandalios, 1993).

In general, it has been proposed that the resistance to oxidative stress obtained from mild stress was correlated with an increased activity of antioxidant enzymes (Scandalios, 1993). Our study also provided evidence supporting this fact.

Changes of Total Activity and Isozyme Pattern on Non-Denaturing Gel of Antioxidant Enzymes by Various Concentrations of Al

The activity of antioxidant enzymes examined in this study constantly increased at the lower Al concentrations and then gradually decreased in higher Al concentration. On the basis of the obtained results, we reasoned that the naked barley cultivar used in this study raised the enzyme level as a defense mechanism in the lower Al concentrations but this was not effective in the higher Al concentration.

The increased antioxidant enzyme activity has already been examined in various stresses such as heavy metal (Cho and Park, 1999), herbicides (Kang et al., 1999), salt, chilling, and wounding. According to the results on growth, this naked barley cultivar could endure the lower Al concentrations but could not endure the higher Al concentrations. So although an increase in activities of antioxidant enzymes occurred, it was thought that the changes in activities of antioxidant enzymes observed in this experiment might not be enough to block the severe Al stress. However, the fact that the change of growth pattern at various Al concentrations coincided with the change of antioxidant enzyme seemed to be notable, because the results suggested that the growth reduction may be related to the changes of antioxidant enzymes and furthermore it may be caused by an oxidative stress.

It suggested that GPX may respond to the Al stress more sensitively than other peroxidases such as FPX and DABPX or that not all of the POX isozymes are increased by Al stress.

Changes of Total Activity and Isozyme Pattern on Non-Denaturing Gel of Antioxidant Enzymes by 100 μM of Al for 48 h

The fact that not all of POX isozymes examined in this study exhibited a similar pattern suggested that not all of the POX isozymes are increased by Al stress and GPX is more important than other POX isozymes. Asada (1992) reported that the role of GPX in the removal of H_2O_2 might be critical in metal induced oxidative stress, because of a significant increase in GPX activity and strong qualitative metal specific changes in the GPX isozyme pattern. Cho and Park (1999) also reported that GPX activity appeared to be expressed during longer exposure to the metal. Results obtained from this study strongly support their opinions.

Kang et al. (1998) suggested that CAT is easily deactivated under the severe stress. The results obtained from this study were opposed to their opinion. Our data suggested that CAT's role may also be critical as other antioxidant enzymes under the severe stress.

Many scientists have emphasized the importance of APX in connection with ascorbate-glutathione cycle which is mainly proceeded in chloroplast and cytosol (Prasad, 1997; Kang et al., 1998) and sug-

gested that this enzyme is more abundantly expressed than other antioxidant enzymes under the severe stress. However, the change of this enzyme shown in this study was not so conspicuous and it merely showed the change in a similar level to other antioxidant enzymes. The results suggested the possibility that either this enzyme was not extracted well since it is mainly located in the chloroplast or ascorbate-glutathione cycle did not function properly under the Al stress.

Concerning GR, because only the total activity was measured by spectrophotometer in this study, the examination on GR isozyme pattern will be needed in the next study.

In summary, the activity of antioxidant enzymes increased at lower Al concentrations and then gradually decreased in higher Al concentrations. Their activity also increased when the exposure time to Al was extended. Changes in the isozyme patterns of antioxidant enzymes more or less coincided with the changes of their total activities. Changes in the total activities of antioxidant enzymes also coincided with the changes of the root growth. Similar results were also obtained in the experiment when other naked barley cultivar, Naehanssalbori and corn, Golden Cross Bantom were used. Since a growth reduction in the roots by Al stress could be related with the changes in the activities of antioxidant enzymes, these results suggested that Al may cause the oxidative stress in the roots of this naked barley cultivar.

There are many reports that correlate antioxidant enzymes or genes with oxidative stress. Richards et al. (1998) reported that the transcripts of oxidative stress genes including POX and CAT gene were significantly accumulated in response to Al stress. Scandalios (1993) reported that such environmental conditions as drought, chilling, anoxia and pathogenic injury have been correlated with antioxidant enzyme activity. Krupa et al. (1993) reported that the high sensitivity of plants to metal-induced phytotoxicity is thought to be due to inhibitory effects on antioxidant enzyme activities. Rao et al. (1996) also reported that oxidative stress, which induced changes in total activities of antioxidant enzymes, could be due to changes in their enzyme content and/or the synthesis of new isozymes.

Recently, studies on this topic have been progressing actively all over the world. However, while the most recent studies on Al stress have been focused either at the genetic or morphological level, research connecting Al stress with oxidative stress at the enzyme level has been relatively neglected. The aim of this study was to characterize the effects of Al stress at the enzyme level, and it suggested that Al could cause the oxidative stress.

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