Effect of aluminum on the isozymes of the seedlings of two soybeans [*Glycine max* (L.) Merrill] varieties

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ABSTRACT

Aluminum (Al) is not the necessary nutrient but the most abundant metal in the earth's crust. Isozymes of the seedlings of two soybean varieties (Zhechun No. 3 and Huachun No. 18) and their response to different Al treatments had been analyzed. The study showed that the responses to Al toxicity of different isozymes were quite different. Among these four isozymes, esterase (EST) isozyme was the least sensitive to Al toxicity. There was no distinct difference in the zymograms of EST under different Al treatments. Al toxicity enhanced the activity of peroxidase (POD) and induced some new bands to adapt it. Superoxide dismutase (SOD) was inhibited by Al toxicity and showed the descending of the activity and disappearing of some bands. The cytochrome-oxidase (COD) activity was also enhanced, but the enhancement of the activity of POD and COD occurred under the treatments of low Al concentration. With the increasing of Al concentration, too much Al disturbed the expression of POD and COD isozymes, which showed that the activity of those isozymes descended. Furthermore, there was no consistence with the response to Al treatments among different bands of the same isozymes. Besides, there was some genotypic variation between two soybean varieties. The bands of these isozymes varied differently between two soybean varieties under the same Al treatments. Compared with Huachun No. 18, Zhechun No. 3 had a stronger tolerance to Al stress relatively.

Keywords: aluminum; polyacrylamide slab gel electrophoresis; seedlings; soybean

Aluminum (Al) is the most abundant metal in the earth's crust and occurs in a number of different forms in the soil. Naturally, Al is mostly found as oxide or silicate precipitates that are not toxic to plants, but the low pH could increase the Al-solubilization and Al speciates to a soluble octahedral hexahydrate form (commonly called Al^{3+}, a phytotoxic ion), which is believed to be the primary phytotoxic Al species (Moffat 1999). Al toxicity is manifested in acid condition. In acidic soils, Al is the major constraint for plant growth and crop production, currently destroying more than 40% of agricultural land around the world (Von Uexkull and Mutert 1995) for example that Al toxicity is a limiting factor in wheat production in the wheat belt of Western Australia.

Al toxicity in plants is often clearly identified by morphological and physiological methods especially in Al-sensitive plant species. Al rapidly inhibits root elongation and prevents development of the ramified root system, an essential feature for successful plant development. The root system as a whole is coralloid in appearance with many stubby lateral roots but lacks fine branching and the lateral roots become stubby, thicken and turn brown (Roy et al. 1988). The reduction of the root system also makes sensitive to other abiotic stress, such as water and nutrient stress, and ultimately reduces crop yield (Von Uexkull and Mutert 1995, Matsumoto 2000, 2002, Matsumo et al. 2001). Al toxicity affects the uptake, transport and also use of several essential nutrients (Ca, Mg, K, P and Fe). Excess Al even induces iron (Fe) deficiency symptoms in rice, sorghum and wheat (Clark et al. 1981, Furlani and Clark 1981, Foy and Fleming 1982). At the molecular level, Al generally inters

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with cell division in root tip and lateral roots, increases cell wall rigidity by cross linking pectin, reduces DNA replication by increasing the rigidity of the DNA double helix, fixes phosphorous in less available form in soils and on root surfaces, decreases root respiration, interferes with enzyme activity governing sugar phosphorylation and the desertion of cell-wall polysaccharide (Foy 1992). In addition, several studies have focused on the plasma membrane functions, while the potential apoplastic and symplastic Al target sites in plant cells are under debate (Horst 1995, Rengel 1996). Al toxicity can cause an excessive generation of toxic reactive oxygen species, a diminished detoxification capacity of these harmful oxygen species and an increase in the peroxidation and breakdown of membrane lipid (Cakmak and Horst 1991). Renato and Paulo (1997) also found negative effects of Al on the nitrate reductase activity (NRA), which is the first enzyme involved in the NO_3^- assimilation in plants. Prolonged Al stress induced an enhancement of lipid peroxidation and caused the formation of highly toxic oxygen free radicals. An increase in the activities of superoxide dismutase and peroxidase and a decrease of catalase activity indicates the presence of an antioxidant scavenging system in Al-treated roots (Renato and Paulo 1997). However, several hypotheses have been suggested for Al toxicity mechanisms until now and are under debate presently (Horst 1995, Taylor 1995, Matsumoto 2000). The mechanism of Al toxicity in plants, especially at the molecular level, still remains poorly understood. There are little reports about the mechanism of Al toxicity in plant at molecular level.

Enzyme is the product of the gene expression. The isozyme is the basic analytical tool to study the molecular mechanism. As an important genetic marker, it could be used to detect the inheritance and variance among different species or different cultivars with species at the molecular level. It also could be as a biochemical marker to study the tolerance of plant to stress. The variation of zymograms could indicate the sequence and tissue specificity of genetic expression during the course of plant growth to some extent.

This kind of analytical method can be as not only an accurate index to know genetic gene, but also a biochemical criterion to know the genetic expression. It is formed to adapt the requirements of cellular metabolism during the course of organic evolution. To some extent the variation of zymogran can indicate the sequence of genetic expression and tissue specificity in the course of plant growth. As one of the important approach to study contemporary genetics and physiological growth, isozyme analysis is a tool to know the existence and expression of the gene. Compared with morphological characters, it has many advantages such as good repeatability, co-dominant inheritance and a directly indication to gene regulation (Shen and Zhuan 2001). Many studied have been carried out to study the isozymes of plant correlated with their tolerance to disease and stress by isozyme analysis. Li et al. (1998) have studied the relationship between POD isoenzyme and the tolerance of soybean to *Cerospora sojia* Hara and found that the isoperoxidase activity of susceptible variety and resistant variety were both increased, but the activity of resistant variety was higher than that of susceptible variety. The results of Sun et al. (2003) showed that the activities and isozyme bands of SOD, POD and CAT all changed under water stress, among which the changes of bands and activities of peroxidase isozymes were more significant than others. There were few reports about the effect of Al on the isozyme. Zhou et al. (2001) studied the effects of Al on maize and reported that there was significant difference in the roots of Maize under Al stress, especially the band (Rf = 0.4) and the band (Rf = 0.7). The intensity of bands was much correlated with the growth of roots. The results of Xiao and Shen (1999) showed that the SOD activity was significantly descended in mungbean under Al stress. There is no systematic study examining the effect of Al on the isozymes (COD, CAT, SOD and EST) of soybean. In order to make clear the biochemical mechanism of enzyme variability, more studies should be carried out to reveal the relation between the mechanism of Al stress and the variability of enzymes.

In this study, the effects of Al on the isozymes of the seedlings of two soybean varieties were studied by isozyme assay. By analyzing the difference of electrophoretograms under different Al treatments, we studied the correlation between different Al concentration and the four isozymes (POD, SOD, EST and COD) of the antioxidant system in the plant.

MATERIAL AND METHODS

Plant materials. Soybean [*Glycine max* (L). Merrill] seeds of two cultivars, Zhechun No. 3 and Huachun No. 18, used for the study were obtained from the seeds store, Zhejiang Academy of Agricultural Science, Hangzhou, China.

Plant growth condition. The seeds of soybean were selected, disinfected with 0.01% (w/v) potassium permanganate solution for 5 min and washed thoroughly with de-ionized water. Then the seeds were soaked in de-ionized water for 2 hours and germinated in white salvers that filled with clean silica sand at ambient temperature. The seedlings were irrigated by Hoagland's nutrient solution treated by different Al concentration every day. After Al treatment for 6 days, the seedlings were harvested to isozyme assay.

Al treatments. The experiment remained for 6 days with 5 treatments (CK and 4 different Al treatments). CK was the Hoagland's nutrient solution controlled without Al and in a condition of no external Al added. The four treatments were applied with various amounts of Al which was added by aluminum sulfate [$Al_2(SO_4)_3.18\ H_2O$]. We added different quantity of external Al^{3+} into the Hoagland's nutrient solution and made the Al^{3+} concentration to 80, 400, 2000 and 10 000 mg/l.

Preparation of enzyme solution. The seedlings were washed with de-ionized distilled water, dried by filter papers and removed cotyledons. Then the seedlings were ground in Tris-glycine buffer (pH 8.3) in proportion to 1 g/ml and homogenized in an ice bath. The homogenate was centrifuged at 12 000 r/min at 4°C for 15 min. The supernatant was used for the analysis of POD, EST and COD isozyme. The other seedlings were cleaned with de-ionized distilled water and removed cotyledons, then ground in five volumes of 0.05 mol/l cooled phosphate buffer [0.1 mmol/l EDTA, 0.3% (w/v) Triton X-100, 4% (w/v) PVPP, pH 7.8], and homogenized in an ice bath. The homogenate was filtered by gauze and centrifuged at 10 500 g at 4°C for 20 min. Then the supernatant was collected for SOD isozyme assay.

Polyacrylamide gel electrophoresis (isozyme assay). The isozyme assay was used by vertical polyacrylamide slab gel electrophoresis (horizontal discontinuous polyacrylamide gel electrophoresis). Gels were prepared according to the method of Arulsekar and Parfitt (1986) and staining was done according to methods given by Vallejos (1983). The concentration of the gradient gel was varied discontinuously, using 2.5% slab as concentrating gel and 7.5% slab gel as separating gel. The electrophoresis was processed according to the method of Hu and Wan (1985). Sample (20 µl) was applied to each slot of the gel. Electrophoresis condition was set at 15 mA/gel for concentration and 30 mA/gel seperating. Bromophenol blue (BPB) was used as a front marker. When BPB reached the gel end, the electrophoresis was finished. After electrophoresis, slab gels were rinsed with cold distilled water and stained for isozymes by applying standard histochemical methods. There were appropriate staining mixtures for the activity staining of various enzymes. The gels were stained by vinegar-benzidine solution for POD (Sekine et al. 1994), the solution of vinegar-α-naphthoic acid with Fast blue salt RR for EST (Xu et al. 2002), the solution of 4-amino-N, N-dimethylaniline with α-naphthol for cytochrome-oxidase (COD) (Hu and Wan 1985) and Nitro blue tetrazolium solution for SOD (Luo and Wang 1984, Tang 1999). The gels were scanned and recorded by THETMAL IMAGING FTI-500 and analyzed by THETMAL IMAGING FTI-500.

RESULTS

Peroxidase isoenzyme (POD)

Peroxidase is one of the most efficient and widely investigated anti-oxidase systems in plants. It has many heteronyms and has been found highly variable in higher plants (Lu and Jia 1996). POD is an important enzyme that is closely correlated with the growth of plant. It is sensitive to the variation of external environment. The changes of POD isozyme bands can be used as the indicator of the growth of plants.

The Figure 1 showed the POD electrophoretogram of two soybean genotypes under different Al treatments. The activities of most isozyme bands with 80 mg/l Al^{3+} treatment were the highest in the electrophoretogram. For Zhechun No. 3, the activities of band 3, 4, 5, 9 and 14 were higher under 80 mg/l Al^{3+} treatment than CK (no external Al^{3+} added), but with the increasing of Al^{3+} concentration, the activities of these bands descended gradually. The bands of 13 and 15 were induced by 80 mg/l Al^{3+} treatment and not found under other Al treatments. The band of 5 only existed in CK and 80 mg/l Al^{3+} treatment and not found in other treatments, and the isozyme bands of 6, 7 and 8 were found in the other treatments. The activities of band 9 and band 12 also increased under different Al treatments but no regulation was showed.

Compared with Zhechun No. 3, Huachun No. 18 had much more isozyme bands with higher activity, especially under the treatments of 400 mg/l Al^{3+} and 2000 mg/l Al^{3+}. Under these two Al^{3+} concentrations, band 4 and band 17 were induced

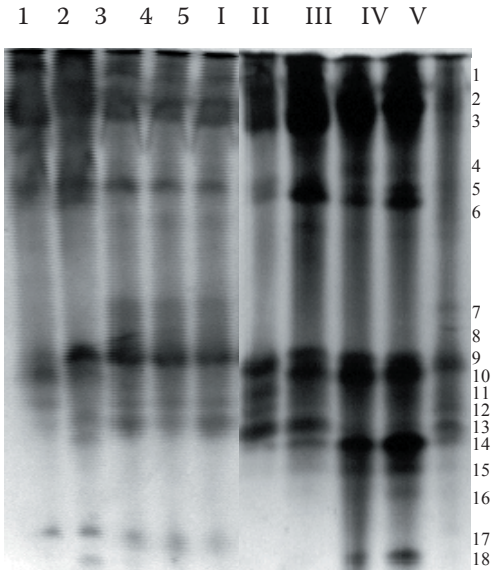

Figure 1. PAGE zymogram of POD isozyme with different Al concentration treatments in seedlings of two soybean cultivars; lanes 1–5 were the PAGE patters of Zhechun, the Al^{3+} concentration is 0, 80, 400, 2000, 10 000 mg/l; lane I–V were the PAGE patters of Huachun, the Al^{3+} concentration is 0, 80, 400, 2000, 10 000 mg/l

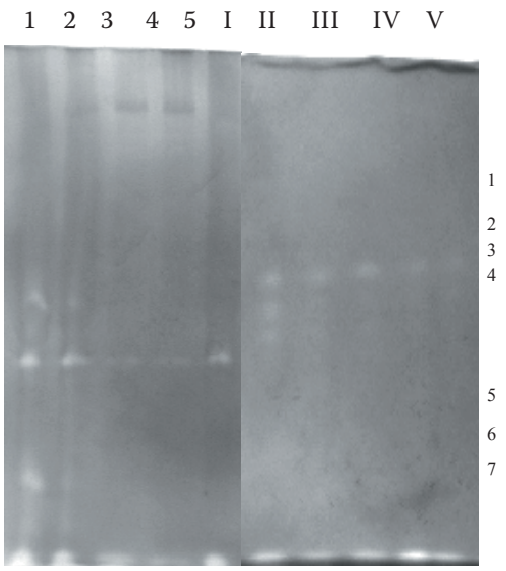

Figure 2. PAGE zymogram of SOD isozyme with different Al concentration treatments in seedlings of two soybean cultivars; lanes 1–5 were the PAGE patters of Zhechun, the Al^{3+} concentration is 0, 80, 400, 2000, 10 000 mg/l; lane I–V were the PAGE patters of Huachun, the Al^{3+} concentration is 0, 80, 400, 2000, 10 000 mg/l

with higher activities along with the increasing of Al^{3+} concentration. Nevertheless, these two bands disappeared and band 8, 9 were induced under 10 000 mg/l Al^{3+} treatment. The other bands were conservative and clearly presented in all treatments. Among these, the descendent of band 11, 12, 13 and 14 were more obvious with the increasing of Al concentration. The activities of the bands of 1, 2, 3, 5, 6 and 11 increased with the increasing of Al^{3+} concentration and only decreased by 10 000 mg/l Al^{3+} treatment. The bands of 2, 3, 5 and 6 had the highest activity under 80 mg/l Al^{3+} concentration, but the activity of band 1, 10 and 11 had its highest value under 400 mg/l Al^{3+} and 2000 mg/l Al^{3+}.

Generally, the zymograms showed that it had much more number of isozyme bands with stronger activity under different Al treatments than no external Al^{3+} added. The results of Lu and Jia (1996) showed that the increasing of POD may be one of the physiological adaptability of plant cell to resist to environmental stress and enhanced its cellular metabolism. The descendents of the bands' activity under the treatments of high Al^{3+} concentration indicated that too much Al^{3+} would harm the plant cellular metabolism too greatly to recover.

Superoxide dismutase isozyme (SOD)

Superoxide dismutase (SOD) is one enzyme of the antioxidase system and generally exists in all kinds of plants. The isoforms of SODs are quite different. Three classes of SODs differing in the metals at their catalytic active site are known in plants (Yao et al. 1994). SODs are ubiquitous metalloenzymes that catalyze dismutation of superoxide radicals and prevents organisms from oxidative damage of too much oxygen free radicals, especially the damage to cytoplasm membrane. To some extent, the activity of SODs in adversity may be as an index to evaluate the resisting capacity of organism to adversity.

The white bands represent SOD isozymes and the depth of the color revealed its activity. The Figure 2 showed that the zymograms of two soybean varieties were quite different with their different responses to Al toxicity. Zhechun No. 3 had 6 isozyme bands and Huachun No. 18 had 4 bands in CK. Along with the increasing of Al concentration, the number of bands was decreased obviously and the activity was also descended. Many bands disappeared and no new bands appeared. The isozymes bands of Zhechun No. 3 did not disappear under 80 mg/l Al^{3+} treatment, but their activities

were markedly weakened. When the concentration of Al^{3+} was increased to 400 mg/l, bands 2, 3, 5 and 6 were not found in the electrophoretogram. The activity of band 4 and 7 descended with the increasing of Al^{3+} concentration, but the activity of band 7 increased when the Al^{3+} concentration was 10 000 mg/l Al^{3+}.

The SOD activity of Huachun No. 3, had only 4 active bands, was weaker and more sensitive to Al treatment than that of Zhechun No. 18. The band 2 and band 3 disappeared only under the treatment of 80 mg/l Al^{3+}. The activities of band 1 and band 7 descended markedly with the increasing of Al^{3+} concentration, but the activity of band 7 increased under 2000 mg/l Al^{3+} treatment and descended under the treatment of 10 000 mg/l Al^{3+}.

As a group, the descending of SOD isozymes under Al treatments was rather similar to that of POD isozymes. In the electrophoretogram of SOD isozyme, there was significant difference between the Al treatments and CK. The SOD isozymes of soybean seedlings were relatively sensitive to Al treatments than POD isozymes and the activity of SOD isozyme was dropped quickly with increase of Al. The expression of some SOD isoforms was inhibited even under the treatments of low Al^{3+} concentrations. Beside these, two soybean genotypes had different responses of SOD to Al toxicity. Compared with Huachun No. 18, Zhechun No. 3 had more number of SOD isoforms with higher activity and relatively stronger tolerance to Al toxicity than Huachun No. 18.

Esterase isozyme (EST)

Esterase is a group of enzymes that catalyze the hydrolysis of ester compounds. It can catalyze the hydrolysis and synthesis of the esters of carboxylic acids and take part in the metabolism of esters. Some people think that it may be correlated with the development of tunica intimae system. Moreover, esterase also takes part in the modification, activation and inactivation of many kinds of enzymes (Wu and Tan 2002). Esterase is one kind of monomeric isozymes and one band in the electrophoresis represents a gene expressing product. The different patterns of electrophoretic iosenzyme are always correlated with the difference of relative gene expression. To some extent analyzing the changes of EST isozymes would reveal the effect of outside surroundings to the growth of plant.

The Figure 3 showed that the EST zymograms of two soybean genotypes changed significantly under different Al treatments. The activity and numbers of isozyme bands were decreased at different levels of Al^{3+} concentration. Compared with CK, Zhechun No. 3 had more isozyme bands and higher EST activity than Huachun No. 18 under Al treatments. The band 7 and band 8 were weakened with the increasing of Al^{3+} concentration. The activity of band 9 under the treatment of 400 mg/l Al^{3+} was the highest, but it decreased under 10 000 mg/l Al^{3+} treatment and 2000 mg/l Al^{3+} treatment. No significant changes were detected under other Al treatments.

The electrophoretogram of Huachun No. 18 showed that there was distinct difference between different Al treatments and CK. Under Al treatments, both the number and activity of some isozyme bands were apparently increased. The band 6 and band 9 were both induced by Al treatments. The band 6 was induced by the treatments of 400 mg/l and 2000 mg/l Al^{3+}, but disappeared under 10 000 mg/l Al^{3+} treatment. It showed that the band 6 could be induced by Al, but would be inhibited by too much Al^{3+}. The band of 9 only existed under the treatments of 2000 mg /l Al^{3+} and 10 000 mg/l Al^{3+}, and it did not exist in the zymoprams of other Al treatments and CK. It could be inferred that the band 9 may be correlated with Al toxicity and induced only by high Al concentration. The results of electrophoretograms showed there was no obvious difference between two soybean genotypes under different Al treatments, especially in the electrophoretogram of Zhechun No. 3. It also indicated that EST isozyme was not very sensitive to Al toxicity.

Cytochrome-oxidase (COD) isozyme

Cytochrome oxidase is a marker enzyme of the intimae mitochondrion. As the terminal enzyme in the electronic transport chains of respiration, it is directly correlated with the producing of ATP. The electrophoretogram of COD would vary as the condition of surrounding changes. The results of electrophoresis indicated that the zymograms of two soybean genotypes were quite different under different Al treatments.

The Figure 4 showed that the activities of isozymes in two soybean genotypes both increased with the increasing of Al concentration. Four main bands (the bands of 6, 9, 10 and 11) and some faint bands were observed in the electrophoretogram of Zhechun No. 3. The band 6 and band 10 were faint bands under the treatments of CK and

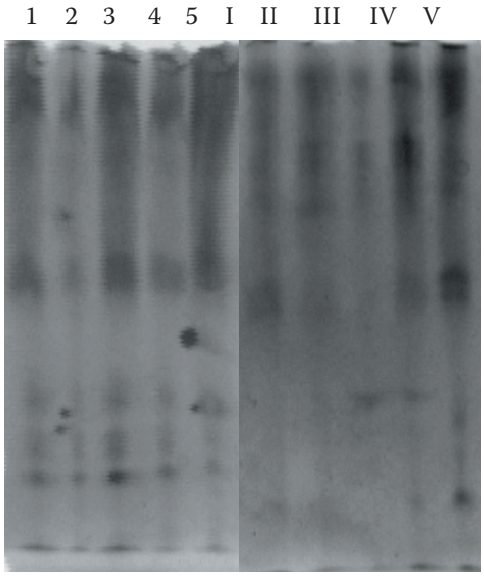

Figure 3. PAGE zymogram of EST isozyme with different Al concentration treatments in seedlings of two soybean cultivars; lanes 1–5 were the PAGE patters of Zhechun, the Al^{3+} concentration is 0, 80, 400, 2000, 10 000 mg/l; lane I–V were the PAGE patters of Huachun, the Al^{3+} concentration is 0, 80, 400, 2000, 10 000 mg/l

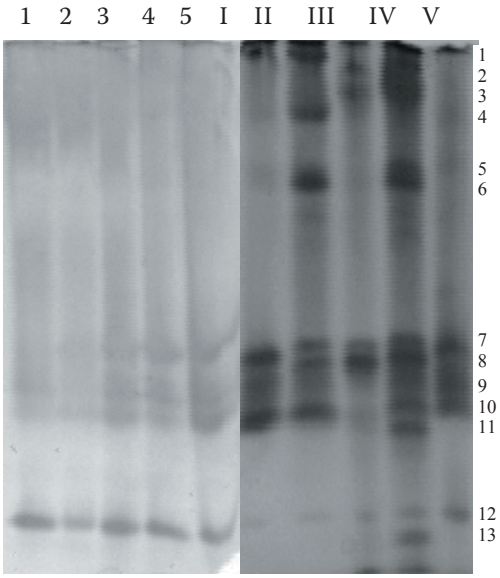

Figure 4. PAGE zymogram of COD isozyme with different Al concentration treatments in seedlings of two soybean cultivars; lanes 1–5, the PAGE patters of Zhechun, the Al^{3+} concentration is 0, 80, 400, 2000, 10 000 mg/l; lane I–V: the PAGE patters of Huachun, the Al^{3+} concentration is 0, 80, 400, 2000, 10 000 mg/l

80 mg/l Al^{3+}. Along with the increasing of Al^{3+} concentration, the activity of these bands also increased, especially for the band 10. The band 10 showed the highest activity under 2000 mg/l Al^{3+} treatment. The band 9 was not detected in the zymograms under CK and 80 mg/l Al^{3+} treatment, but was induced by 400 mg/l Al^{3+} treatment and increased its activity with the increasing of Al concentration. As to the band 11, there was no significant difference in the zymogram under different Al treatments.

Compared with Zhechun No. 3, Huachun No. 18 had much more bands with higher activity. The bands of 1, 6, 7 and 10 were conservative and clearly presented under all treatments. However, the other bands changed differently under different Al treatments. The band of 8 was induced by 10 000 mg/l Al^{3+} treatment. The bands of 11 and 13 were induced by 2000 mg/l Al^{3+} treatments.

DISCUSSION

As the product of the gene expression, isozyme could be a biochemical criterion to know the tolerance of plant to stress and detect the inheritance and variance at the molecular level. In recent years, many studies had shown that isozymes of plant correlated with their tolerance to disease and stress closely. The results of this experiment showed that there was markedly difference between Al treatments and CK in the electrophoretograms between two soybean varieties.

The isozymes of POD and SODs protect the organism in cooperation with each other. The results of our experiment showed that the isozyme patterns of POD and SOD changed dissimilarly under the Al treatments. Generally, it had much number of POD bands with stronger activity under different Al treatments than no external Al^{3+} added. It is possible that POD, as one kind of adaptable enzymes, can enhance the expression of some bands or induce some new POD bands to adapt critical adversity. Lu and Jia (1996) also inferred that the increasing of POD may be one of the physiological adaptability of cell to resist to the environmental stress and enhanced its cellular metabolism to some extent. The results of Zhou et al. (2001) showed two POD isozyme bands, Rf = 0.4 and Rf = 0.7, which changed typically under Al stress. The intensity of these two bands was much correlated with the growth of roots. We found that the activities of most POD isozyme under the treatment of 80 mg/l Al^{3+} were the highest in the electrophoretogram of Zhechun No. 3. The interpretative reason was that Al treatments of low concentrations could increase

plant growth or induce some desirable effects. Some people found the similar results (Foy and Fleming 1982, Foy 1983, 1992), but the activities of POD isozymes descended under the treatments of high Al^{3+} concentration such as 2000 mg/l and 10 000 mg/l. It could be presumed that too much Al^{3+} disturbs the cellular metabolism greatly and the disturbance is not reversible. Some new POD isozyme bands induced by the treatments of high Al concentration may be corrected with Al toxicity. The change of SOD isozymes under Al treatments was quite different to that of POD isozymes. The activity of SOD decreased along with the increasing of Al concentration. To finite Al concentration, some SOD bands were already inhibited and disappeared. The expression of some SOD isozym bands was inhibited and the activity descended under the treatments of low Al concentrations. It indicated that the SOD isozyme of soybean seedlings was relatively sensitive to Al treatments than POD isozyme. When the content of external Al^{3+} added increased to finite concentration, the cellular metabolism was seriously injured and the organism can not recovery from it by itself, which showed that the capability to delimitate superoxide radicals descended. It was the same as the result of Xiao and Shen (1999). From the difference between two soybean genotypes, the POD and SOD isozyme of Huachun No. 18 had much more bands with stronger activity than that of Zhechun No. 3, but the sensitivity to different Al treatments of Zhechun No. 3 was fainter and changes lesser than that of Huachun No. 18. It could infer that the tolerance to Al toxicity of Zhechun No. 3 was stronger than that of Huachun No. 18.

Esterase is a kind of enzyme encoded by DNA. It may be as a marker to class the genetic diversity among colony or internal species. The variety of esterase in organism is the response of gene and a series of interior physiological processes. Esterase mainly takes part in the catabolism of plant and is correlated with phosphorus metabolism. Furthermore, it was reported that EST may be having transformation and can hydrolysis large numbers of esters existing non-physiologically (Bai et al. 1996). The results of our experiment showed that there was no distinct difference among different Al treatments and between two soybean genotypes in the EST electrophoretograms, especially Zhechun No. 3. The EST isozyme was stable and not sensitive to Al toxicity. It indicated that EST seemed not fit to be as an index to study plant stress physiology. In fact, it used to be as an analysis tool to analyze the genetic differences between species and among cultivars with species (Dhiraj et al. 2003, Kenichi et al. 2003).

Cytochrome (Cyt) oxidase is the terminal enzyme in the electronic transport chains of respiration in all high plant. It is the key of the electronic transport chains and catalyzes the step of transferring electron from Cyt C to O_2, which is the most basic nonreversible step in energy metabolism and have an important position in the respiration. The results of our experiment showed that the activities of COD isozyme bands of two soybeans were increased along with Al treatments to some extend. Huachun No. 18 had more bands than Zhechun No. 3. Both of them had been induced some new bands under different Al treatments. We presumed that the organism had to enhance its respiration, which showed the increase of the activities of COD isozyme, to decrease the harm produced by Al and remain the balance of cellular metabolism in soybean seedlings. The genetic difference of COD electrophoretograms between two soybean varieties was similar to that of POD and SOD, Zhechun No. 3 was injured less and had stronger tolerance to Al toxicity than Huachun No. 18.

On the whole, Al treatments had influenced the 4 kinds of enzymes to some extent, but there were some difference among these 4 enzymes responding to Al toxicity. It based on the sensitivity of the enzyme to Al stress. The Al treatments enhanced the activity of POD enzyme and induced some new bands, but the expression of SOD enzyme was inhibited and the activity was decreased. However, the effects of Al on the EST and CYT were not as distinct as POD and SOD. Furthermore, there was no consistence with the response to Al treatments among different bands of the same enzyme. To analysis this, we think that it was the result of cellular differentiation. The different enzyme bands in cell were produced to adapt to different cellular circumstance. At the same adversity, the variability of one enzyme band may be isochronous with another, even reverse. The response of soybean seedlings to Al toxicity was complicated.

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