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REGULAR ARTICLE

The accumulation and transport of abscisic acid in soybean (*Glycine max* L.) under aluminum stress

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Abstract Abscisic acid (ABA) plays an important role in mediating some biotic and abiotic stresses. In the present study, to better understand the relationship of ABA production and Aluminum (Al)-resistance in plants, Al-resistance genotype (Jiyu70) of soybean was adopted to investigate the accumulation and transport of ABA in plants exposed to Al. Results showed that exogenous application of ABA and ABA synthesis inhibitor-fluridone respectively increased and reduced endogenous ABA content in root apices of soybean, and results in the corresponding reduction and aggravation of Al toxicity. Increasing of either Al concentration (0–50 μ*M*) or treatment duration (0–12 h, 30 μ*M* Al) cause a higher inhibition of root elongation and ABA accumulation in root apices of soybean. Al-induced enhancement of endogenous ABA production not only was in roots but also in leaves, whereas La^{3+} (behaves similarly as Al^{3+} at the level of cell surface) only increased ABA accumulation in roots. In split-root experiments, Al treatment in two parts of roots (Part A, + Al; Part B, + Al) both decreased root elongation and increased ABA accumulation in root apices of soybean. Whereas when only part A of roots was exposed to Al (Part A, + Al; Part B, -Al), endogenous ABA content in root apices increased in part A but inversely in part B, but root elongation inhibition only was found in part A. Using [^{3}H]-ABA radioisotope technique, it was found that [^{3}H]-ABA can transport at the rate of more than 3.2 cm·min^{-1} in the whole plants, and this can be accelerated by Al supply. In addition, [^{3}H]-ABA tended to distribute in the root part under Al stress. Together, these results suggest that ABA may play an important role in regulating Al resistance of soybean as an Al-stress signal.

Keywords Aluminum toxicity · Soybean · Abscisic acid · Root elongation

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Introduction

When soil becomes acidic as a result of natural processes or human activities, Al is solubilized as toxic trivalent cation, Al^{3+}. Al toxicity is considered to be one of the most important limiting factors of crop

production on acid soils (Kochian et al. 2004), which can rapidly inhibit plant root growth, subsequently decrease nutrient and water acquisition, and results in poor crop growth and productivity (Kochian et al. 2004). Root apex is a critical site of perception and expression of Al toxicity (Ryan et al. 1993; Vázquez et al. 1999), which has been reported in beans (Rangel et al. 2007), wheat (Ryan et al. 1993) and maize (Sivaguru and Horst 1998). However, how Al induced inhibition of root growth is still ambiguous.

Several studies have been reported that phytohormones play an important role on Al toxicity and tolerance mechanisms. In *Phaseolus vulgaris* L., Al can increase the accumulation and change the composition (particularly zeatin and dihydrozeatin) of cytokinines in roots during the early stage before inhibition of root growth ((Massot et al. 1994, 2002). Exogenous cytokinines supply can inverse the adverse effect of Al on soybean shoots growth (Pan et al. 1988, 1989). The inhibition of root growth could be due to the rapid increase of cytokinins directly or indirectly affects other hormone homeostasis of plants, for instance, enhances ethylene production in roots of *Phaseolus vulgaris* L. (Massot et al. 2002). However, influence of Al on ethylene has also been studied on maize and it showed that ethylene have no effect on the Al toxicity of maize root (Gunsé et al. 2000). Moreover, Al-induced inhibition of basipetal auxin transport in maize roots has been suggested as an Al toxic mechanism (Kollmeier et al. 2000). Kumari et al. (2008) found that transcripts putatively related to auxin, ethylene and cytokinin metabolism in *Arabidopsis thaliana* were affected by Al. Exogenous application of salicylic acid can decrease Al accumulation in root apex and enhance root elongation by increasing the amount of citrate exudation from roots of *cassia tora* (Yang et al. 2003). In *Medicago truncatula*, gene involved in ethylene biosynthesis, 1-aminocyclopropane-1-carboxylate oxidoreductase was suggested to be involved in Al toxicity and that the down-regulation of this gene might be an essential adaptive response to prevent further inhibition of root growth (Chandran et al. 2008).

ABA is a phytohormone involved in fundamental physiological processes of higher plants, such as response to abiotic stresses (temperature, light, and drought), regulation of seed dormancy and germination, control of stomatal closure (Anderson et al. 1994; Borel et al. 1997; Li et al. 2000). ABA is not only a stress hormone but also an endogenous signal required for proper development. In the absence of environmental stress, a basal ABA level modifies optimal growth of plants possibly by reducing growth-inhibitory ethylene release (Cheng et al. 2002; Sharp 2002). ABA with over certain threshold levels can precipitate the stress-related effects such as complete closure of stomata and massive alteration of gene expression (Hoth et al. 2002). By loading [^{3}H]-ABA in roots of *Vicia faba* L., it was found that ABA rapidly transported from roots to shoots and accumulated in apoplast of guard cells in response to water stress (Jia et al. 1996). The role of ABA as a root-to-shoot stress signal is now well established.

Several studies also indicated that ABA was involved in Al toxicity and Al-resistance mechanisms. Exogenous application ABA could ameliorate Al-induced root elongation inhibition in soybean (Shen et al. 2004). Al treatment increased endogenous ABA content in the roots of maize (Klimashevskii 1983), soybean (Shen et al. 2004) and barley (Kasai et al. 1993a, b). However, Al treatment can not affect the ABA concentration in the xylem sap of sugar maple (*Acer saccharum* Marsh) (Bertrand et al. 1995). Furthermore, studies showed that exogenous ABA supply enhanced Al-induced citrate secretion from roots of soybean (Shen et al. 2004) and caused oxalate exudation from roots of buckwheat in absence of Al treatment (Ma et al. 2001), but did not affect Al-induced malate efflux from roots of wheat (Ryan et al. 2003). Whether and how ABA is involved in Al resistance in plants is still less clear. In the present study, Al-resistance genotype of soybean Jiyu70 was adopted to investigate the accumulation and transport of endogenous ABA under Al stress and its role in Al resistance.

Materials and methods

Plant materials and culture conditions

Seeds of Al-tolerant cultivar (Jiyu70) of soybean were geminated in peat moss for 3 days at 25°C in darkness. After germination, similar seedlings were transferred into one liter plastic pot containing 0.5 m*M* $CaCl_2$ solution (pH 4.5) and pre-cultured for 24 h. Afterwards parts of plants were used for evaluation of Al toxicity by measuring root elongation, the remaining plants were continually cultured in

one liter continuous aerated nutrient solution (Horst et al. 1992) containing (in μ*M*): KNO_3, 750; Ca $(NO_3)_2$, 250; $MgSO_4$, 325; KH_2PO_4, 10; Fe-EDTA, 20; H_3BO_3, 8; $CuSO_4$, 0.2; $ZnSO_4$, 0.2; $MnCl_2$, 0.2; $(NH_4)_6Mo_7O_{24}$, 0.2. The pH of the solution was adjusted to 4.5 with 0.1 M HCl and renewed every three days. All the experiments were performed in a controlled growth chamber under the following conditions: a 14 h/10 h light/dark cycle, 25/20°C day/night temperature, a light intensity of 300 μmol photons m^{-2} s^{-1} and a relative humidity of 70%.

Effect of exogenous ABA and fluridone on root elongation and endogenous ABA accumulation of root apices under Al stress

Four-days-old seedlings were grown in 0.5 m*M* $CaCl_2$ solution (pH 4.5) containing $AlCl_3$ (0 or 30 μ*M*) with or without ABA (5 μ*M*) or fluridone (FLU, ABA synthesis inhibitor) (1 μ*M*) for 24 h. According to the description of Yang et al. (2000), root length was measured by a ruler before and after 24 h treatment. The relative root elongation (RRE) was calculated as: (root elongation in various treatments/root elongation in control) × 100.

Ten-days-old seedlings were cultured in 0.5 m*M* $CaCl_2$ solution (pH 4.5) for 12 h in dark. Then roots of seedlings were treated with 0.5 m*M* $CaCl_2$ solution (pH 4.5) containing $AlCl_3$ (0 or 30 μ*M*) without or with ABA (5 μ*M*) or FLU (1 μ*M*) for 9 h. After treatment, root apices (0–1 cm) were excised and frozen in liquid nitrogen immediately, and stored at −80°C for ABA determination.

Effect of different Al concentrations on root elongation and endogenous ABA accumulation in soybean root apices under Al stress

Four-days-old seedlings were grown in 0.5 m*M* $CaCl_2$ solution (pH 4.5) containing $AlCl_3$ (0, 10, 30, 50 μ*M*) for 24 h. Root length was measured by a ruler before and after 24 h treatment. The RRE was calculated as (root elongation with $AlCl_3$ treatment/root elongation without $AlCl_3$) × 100.

Ten-days-old seedlings were cultured in 0.5 m*M* $CaCl_2$ solution (pH 4.5) for 12 h in dark. Then seedling roots were treated with $AlCl_3$ (0, 10, 30, 50 μ*M*) in 0.5 m*M* $CaCl_2$ solution (pH 4.5) for 9 h. After treatment, root apices (0–1 cm) were excised, and frozen in liquid nitrogen immediately, then stored at −80°C for ABA determination.

Time course of root elongation inhibition and endogenous ABA changes in root apices under Al stress

Four-days-old seedlings were grown in 0.5 m*M* $CaCl_2$ solution (pH 4.5) containing $AlCl_3$ (0 or 30 μ*M*) for 12 h. Root length was measured by a ruler every 3 h, then root elongation were calculated.

Ten-days-old seedlings were cultured in 0.5 m*M* $CaCl_2$ solution (pH 4.5) for 12 h in dark. Then roots of seedlings were treated with $AlCl_3$ (0 or 30 μ*M*) in 0.5 m*M* $CaCl_2$ solution (pH 4.5) respectively for 3, 6, 9, 12 h. Primary root apices (0–1 cm) were sampled in different time courses. The root apices were excised, frozen in liquid nitrogen immediately, and stored at −80°C for ABA determination.

Effect of Al and La treatment on endogenous ABA accumulation in soybean root apices and leaves

Ten-days-old seedlings were cultured in 0.5 m*M* $CaCl_2$ solution (pH 4.5) for 12 h in dark. Then seedling roots were treated with 0.5 m*M* $CaCl_2$ containing $AlCl_3$ (0 or 30 μ*M*) or $LaCl_3$ (0 or 10 μ*M*) for 9 h. After treatment, root apices (0–1 cm) or top two completely unfolded leaves (about 1.0 g) were harvested, frozen in liquid nitrogen immediately, and stored at −80°C for ABA determination.

Split-Roots experiment

After germination, the primary roots of soybean seedlings were excised and then plants were cultured in nutrient solution for 10 days to obtain uniform lateral roots. The lateral roots were split into two equal parts (part A and part B) by the root manipulation box described as Yang et al. (2001) and split roots were washed with 0.5 m*M* $CaCl_2$ solution (pH 4.5) for 12 h in dark. Then: 1) the split roots of one half of plants were separately exposed to 0.5 m*M* $CaCl_2$ solution (pH 4.5) containing $AlCl_3$ (0 or 30 μ*M*), root elongation was measured by a ruler before and after 24 h treatment; 2) The split roots of the other half of plants were separately exposed to 0.5 m*M* $CaCl_2$ solution (pH 4.5) containing $AlCl_3$ (0 or 30 μ*M*) for 9 h. After treatment, root apices (0–1 cm) were

excised, frozen in liquid nitrogen immediately, and stored at −80°C for ABA determination.

Effect of Al on the distribution and transport of ABA in soybean

Ten-days-old seedlings were cultured in 0.5 m*M* $CaCl_2$ solution (pH 4.5) for 12 h in the dark. To determine the transport rate of [^{3}H]-ABA, [^{3}H]-ABA was loaded in soybean roots by exposure to 0.5 m*M* $CaCl_2$ solution (pH 4.5) containing [^{3}H]-ABA with the radiation intensity of 1×10^{-3} mCi ml^{-1} for 5 min. Then soybean seedlings were grown in 0.5 m*M* $CaCl_2$ solution (pH 4.5) for 5 min. The average height of soybean shoots was 16 cm. Shoots were harvested and separated into four parts (0–4 cm, 4–8 cm, 8–12 cm, and 12–16 cm) from basital to top for radioactivity determination.

Ten-days-old soybean roots were split into two equal parts (part A and part B) using the root manipulation box for different treatments. [^{3}H]-ABA was always loaded in part A by subjecting part A to 0.5 m*M* $CaCl_2$ solution (pH 4.5) containing of 1×10^{-3} Ci mmol^{-1} [^{3}H]-ABA. At the same time, Part B was growing in 0.5 m*M* $CaCl_2$ solution (pH 4.5). After 5 min loading, Part A and part B were respectively cultured in 0.5 m*M* $CaCl_2$ solution containing $AlCl_3$ (0 or 30 μ*M*) (pH 4.5). After 2 h treatment, plants were harvested and separated into roots, stems and leaves for radioactivity determination.

Ten-days-old seedlings were cultured in 0.5 m*M* $CaCl_2$ solution (pH 4.5) for 12 h in the dark. Then: 1) Roots were subjected to 0.5 m*M* $CaCl_2$ solution (pH 4.5) containing [^{3}H]-ABA with the radiation intensity of 1×10^{-3} mCi ml^{-1} for 5 min; 2) A smaller cut was made by a blade in the top leaves of plants grown in 0.5 m*M* $CaCl_2$ solution (pH 4.5), then 2 μl [^{3}H]-ABA (1×10^{-3} mCi ml^{-1}) was applied repeatedly to the cut with a small brush for 5 min. After loading, roots were subjected to 0.5 m*M* $CaCl_2$ solution (pH 4.5) with or without $AlCl_3$ (30 μ*M*) for 5 min and 2 h, then roots, stems and leaves of plants were harvested for radioactivity determination. The plant samples were separately trimed and well mixed.

Determination of radioactivity

About 0.1 g samples were weighed and transferred into a scintillation bottle with 1 ml $HClO_4$:H_2O_2 (1:1, v:v) mixture, and digested for 2 h at 80°C. Then 5 ml scintillation solution (TritonX-100:toluene (3:7, v:v), 0.5% (m/v) PPO) was added and kept in the dark for 24 h. Radioactivity in the digestion was measured with Tri-Carb 2800TR scintillater.

Determination of endogenous ABA in soybean

Freeze-dried tissue samples were weighed, homogenized, and extracted with homogenization buffer [80% (*v*/*v*) aqueous methanol +10 mg l^{-1} butylated hydroxytoluene]. The extracts were passed through a C_{18}-reversed phase prepacked column immediately under dim light conditions at 4°C to get rid of xanthoxal. The methanol solution was dried under reduced pressure and the aqueous residue partitioned three times against ethyl acetate at pH 2.5. Ethyl acetate in the combined organic fractions was evaporated to dryness under a N_2 stream and the residue was dissolved in 0.5 ml of TBS-buffer for indirect enzyme-linked immunosorbent assay (ELISA) as described by Mertens et al. (1983) with monoclonal antibodies. The procedure is followed manufacturers recommendations of a commercial kit (Chinese Agriculture University, Beijing, China). Endogenous ABA is determined by an automatic enzyme-linked immunosorbent assay systems (Bio-Tek Elx800).

Statistical analysis

Each result shown in tables and figures was the mean of at least three replicates. The significance of differences between treatments was statistically evaluated by standard deviation and *t*-test methods.

Results

Effect of ABA and FLU on Al-induced root elongation inhibition and endogenous ABA content in soybean roots under Al stress

Measurement of relative root elongation in $CaCl_2$ solution at pH 4.5 is a widely used method for assaying Al tolerance in plants (Yang et al. 2000). In present study, the same screening system and 30 μ*M* $AlCl_3$ were used to evaluate the role of ABA in Al tolerance of soybean. Neither ABA (5 μ*M*) nor ABA synthesis inhibitor, FLU (1 μ*M*) had effect on

the soybean root growth (Fig. 1A). Root elongation was inhibited approximately 50% when roots was exposed to 30 μ*M* $AlCl_3$ during 24 h, whereas when 5 μ*M* ABA was added together with Al, the root growth was inhibited by about 40%. FLU (1 μ*M*) together with Al (30 μ*M*) resulted in about 80% root elongation inhibition (Fig. 1A). It suggested that application of exogenous ABA and FLU respectively ameliorated and aggravated the Al toxicity.

Exogenous application of ABA or FLU respectively increased or decreased the endogenous ABA content (Fig. 1B). Al-induced increasing of endogenous ABA in soybean root apices was enhanced by exogenous ABA (5 μ*M*) application, but was prevented by FLU (1 μ*M*) (Fig. 1B), which was consistent with the response in the Al-induced root elongation inhibition (Fig. 1A).

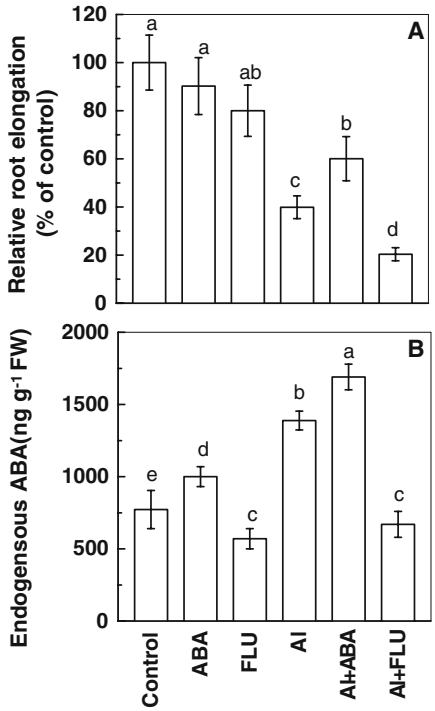

Fig. 1 Effect of ABA and fluridone (FLU) on Al-induced root elongation inhibition (**A**) and endogenous ABA content in soybean roots (**B**). Four-days-old soybean seedlings were exposed to 0.5 m*M* $CaCl_2$ solution (pH 4.5) containing $AlCl_3$ (0 or 30 μ*M*), ABA (0 or 5 μ*M*) or FLU (0 or 1 μ*M*) for 24 h. Root length was measured before and after treatment. RRE were calculated as mentioned in the material and method. Ten-days-old soybean seedlings were exposed to $AlCl_3$ (0 or 30 μ*M*) in 0.5 m*M* $CaCl_2$ solution (pH 4.5) containing ABA (0 or 5 μ*M*) or FLU (0 or 1 μ*M*) for 9 h. Then root apices (0–1 cm) were excised for ABA analysis. Error bars represent SD (n=30). Significant differences among the treatments are indicated by different letters (p<0.05, Tukey test)

Effect of Al concentration and duration of Al treatment on root elongation and endogenous ABA accumulation in soybean root apices

To understand the relationship of Al-resistance and Al-induced endogenous ABA changes, endogenous ABA content and relative root elongation in response to different Al concentration and Al duration treatment were investigated. Al increased the level of endogenous ABA in root apices in dose- and time-dependent manners (Figs. 2B and 3B). The endogenous ABA content increased with the increase of Al treatment concentration (10–50 μ*M*) and particularly in the presence of 50 μ*M* Al with approximately two times more than control (Fig. 2B). The change of Al-induced root elongation inhibition was consistent with endogenous ABA content, and 50 μ*M* Al treatment resulted in about 70% root elongation inhibition (Fig. 2A). Furthermore, time course (3–12 h) experiment showed that the endogenous ABA accumulation in roots exposed to 30 μ*M* Al is obviously higher than that of control (Fig. 3B). The root growth was proportionally inhibited (20%–80% of control) with Al treatment time (3–12 h) (Fig. 3A). These results indicated that there is a negative correlation between endogenous ABA accumulation and root elongation under Al stress.

Comparison of the effect of Al and La on endogenous ABA accumulation in soybean

La^{3+} has similar chemical properties as Al^{3+}. In the present study, 30 μ*M* $AlCl_3$ and 10 μ*M* $LaCl_3$ caused similar root elongation inhibition during 24 h treatment (data not shown). After 9 h Al treatment, the level of endogenous ABA in both roots and leaves increased comparing with that in the control plants (Fig. 4A, B). Whereas compared to Al, La only enhanced ABA content in root apices (Fig. 4A), but not in leaves (Fig. 4B).

Transport and distribution of ABA in soybean tissues

In split-roots experiment, the lateral roots of single soybean seedling were divided into part A and part B for different treatment. When both part A and part B

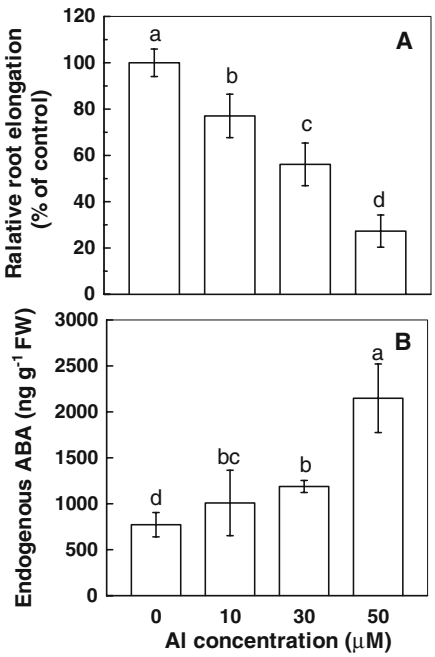

Fig. 2 Effect of different Al concentrations on Al-induced root elongation inhibition (**A**) and endogenous ABA in soybean roots (**B**). Four-days-old soybean seedlings were exposed to $AlCl_3$ (0, 10, 30, 50 μ*M*) in 0.5 m*M* $CaCl_2$ solution (pH 4.5) for 24 h. Root length was measured before and after treatment. RRE were calculated as mentioned in the material and method. Error bars represent SD (n=30). Ten-days-old seedlings were cultured in 0.5 m*M* $CaCl_2$ solution (pH 4.5) overnight. Then roots were treated with $AlCl_3$ (0, 10, 30, 50 μ*M*) in 0.5 m*M* $CaCl_2$ solution (pH 4.5) for 9 h. After treatment, root apices (0–1 cm) were cut, frozen in liquid nitrogen immediately, and stored at −80°C for ABA determination. Significant differences among the treatments are indicated by different letters (p<0.05, Tukey test)

were treated with Al (0 or 30 μ*M*), the results of the root elongation inhibition and endogenous ABA accumulation were consistent with those in whole root experiment. When only part A of roots was treated with Al (Part A, + Al; Part B, -Al), endogenous ABA content in root apices increased in part A but inversely in part B, whereas root elongation inhibition was only found in part A (Fig. 5A). It was suggested that Al may cause a transport and re-distribution of ABA in plants to adapt environmental stress, which was further studied with isotopic tracer technique by using [^{3}H]-ABA.

When [^{3}H]-ABA was loaded in the roots for 5 min, there is almost no [^{3}H]-ABA was detected in the shoots, whereas after further 5 min culture, [^{3}H]-ABA can transport to the top of shoot (12–16 cm) (data not shown). According to the average height (16 cm) of plants, the calculated transport rate of ABA was more than 3.2 cm min^{-1}. Thus, it is suggested that [^{3}H]-ABA can transport rapidly in soybean seedlings.

In split-root experiment, [^{3}H]-ABA fed through part A can be transported to stem, leaves and part B of roots (Table 1). Consistent with the above results, [^{3}H]-ABA tend to transport to the Al-exposed root part. Al treatment in both parts of roots increased [^{3}H]-ABA accumulation in roots. More [^{3}H] ABA kept in part A when part A was under Al stress (72.89% in + Al/-Al treatment compared to 64.93% in -Al/-Al treatment), and more [^{3}H] ABA went to part B when part B was under Al stress (10.37% in -

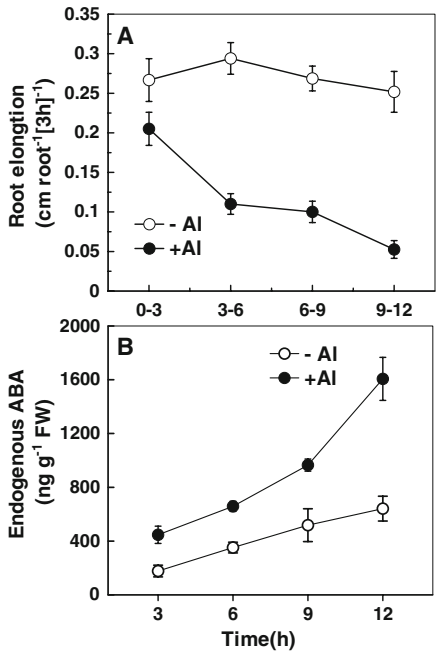

Fig. 3 Effect of Al treatment duration on Al-induced root elongation inhibition (**A**) and endogenous ABA in soybean roots (**B**). Four-days-old soybean seedlings were exposed to $AlCl_3$ (30 μ*M*) in 0.5 m*M* $CaCl_2$ solution (pH 4.5). Root length was measured at 0, 3, 6, 9, 12 h after treatment. Ten-days-old seedlings were cultured in 0.5 m*M* $CaCl_2$ solution (pH 4.5) overnight. Then roots were treated with $AlCl_3$ (0 or 30 μ*M*) in 0.5 m*M* $CaCl_2$ solution (pH 4.5) for 3, 6, 9, 12 h. After treatment, root apices (0–1 cm) were cut, frozen in liquid nitrogen immediately, and stored at −80°C for ABA determination. Error bars represent SD (n=30). Significant differences among the treatments are indicated by different letters (p<0.05, Tukey test)

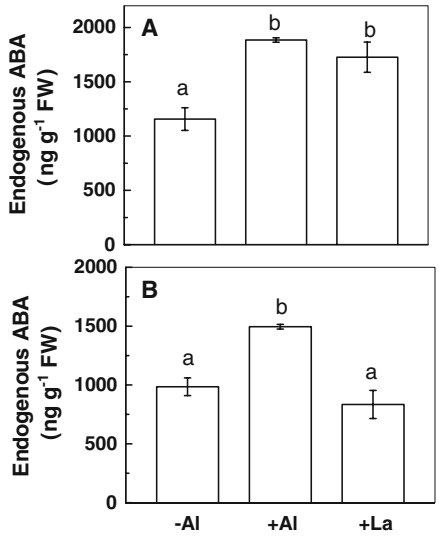

Fig. 4 Effect of Al^{3+} and La^{3+} on endogenous ABA content in roots (**A**) and leaves (**B**) of soybean. Ten-days-old soybean seedlings were exposed to 0.5 m*M* $CaCl_2$ solution (pH 4.5) containing $LaCl_3$ (0 or 10 μ*M*) or $AlCl_3$ (0 or 30 μ*M*) for 9 h. Then root apices (0–1 cm) and leaves (10 mg) were harvested for ABA analysis. Vertical bars represent standard deviation of the mean (n=3). Significant differences among the treatments are indicated by different letters (p<0.05, Tukey test)

Al/+Al treatment compared to 7.43% in -Al/-Al treatment) (Table 1). Al stress enhanced the distribution of ABA in the roots with direct subject to Al stress (Table 1).

Using intact roots, [^{3}H]-ABA loaded in roots free of Al transported rapidly (within 5 min) from roots to top leaves (Table 2), vise versa (Table 3). No matter [^{3}H]-ABA being fed through roots or leaves, the transport and distribution of [^{3}H]-ABA in the roots, stems and leaves was affected by Al treatment. When [^{3}H]-ABA was fed in roots in the absence of Al for 5 min, about 9% [^{3}H]-ABA was detected in the stems and leaves of the control plants, whereas more than 16% [^{3}H]-ABA transported to the shoot in the presence of Al (Table 2), suggesting that Al accelerate ABA transport from roots to shoots. When [^{3}H]-ABA was fed in the leaves for 5 min, more [^{3}H]-ABA was found in the roots of Al-treated plants (Table 3). The results also suggested that Al stress accelerated ABA transport from shoots to roots in soybean (Table 3). After feeding [^{3}H]-ABA from roots or leaves for 2 h, more [^{3}H]-ABA accumulated in the roots under Al stress compared to the corresponding control independent of different feeding site (Tables 2 and 3).

Discussion

ABA is a molecule of multifunction in mediating plant responses to various stresses. It has commonly been regarded as a signal which can transmit drought information when plants suffer drought stress (Verslues and Bray 2006). Moreover, ABA has been implicated in plant response to salinity (Montero et al. 1998), chilling (Anderson et al. 1994), heat (Larkindale et al. 2005) and heavy metals (Hsu and Kao 2004) stresses. In the present study, exogenous application of ABA and ABA synthesis inhibitor FLU respectively increased and reduced endogenous ABA accumulation in roots, and then alleviated and aggravated Al-induced root elongation inhibition in soybean (Fig. 2). Al treatment increased endogenous ABA accumulation in both roots and leaves (Fig. 3), and accelerated ABA transport (Tables 2 and 3). In addition, ABA tended to redistribute in the Al-exposed root part (Tables 1, 2 and 3). All these results suggested that as a signal

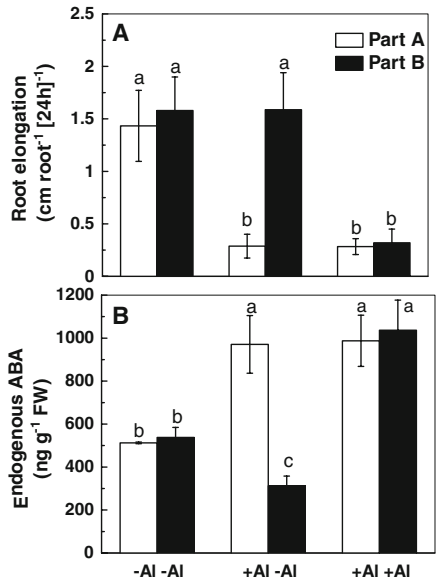

Fig. 5 Effect of Al on root elongation (**A**) and endogenous ABA content in soybean roots (**B**) in split-root experiment. Ten-days-old soybean roots were split into two equal parts (part A and part B) using the root manipulation box. The split roots were separately exposed to 0.5 m*M* $CaCl_2$ solution with or without $AlCl_3$ (30 μ*M*), then 1) after 24 h treatment, root length was measured before and after treatment; 2) after 9 h, root apices (0–1 cm) were excised to analyze the ABA content.. Error bars represent SD (n=30). Significant differences among the treatments are indicated by different letters (p<0.05, Tukey test)

Table 1 The distribution of [^{3}H]-ABA in split root when [^{3}H]-ABA was fed through part A of roots. By using the root manipulation box, 10-days-old soybean lateral roots were split into two equal parts (part A and part B). [^{3}H]-ABA was always loaded in part A by subjecting part A to 0.5 m*M* $CaCl_2$ solution (pH 4.5) containing of 1×10^{-3} Ci mmol^{-1} [^{3}H]-ABA. At the same time, Part B was growing in 0.5 m*M* $CaCl_2$ solution (pH 4.5). After 5 min loading, Part A and part B were respectively treated with 0.5 m*M* $CaCl_2$ solution containing 0 or 30 μ*M* $AlCl_3$ (pH 4.5). After 2 h treatment, plants were harvested and separated to roots, stems and leaves for radioactivity determination.Vertical bars represent standard deviation of the mean (n=3)

Time	Treatment(Part A/Part B)	Radioactivity(dpm%)			
		Root(PartA)	Stem	Leaf	Root(PartB)
2 h	-Al/-Al	64.93±0.41	11.33±0.76	16.31±0.60	7.43±0.57
2 h	+Al/-Al	72.89±0.60	6.83±0.18	15.68±0.56	4.60±1.09
2 h	-Al/+Al	58.43±2.26	10.48±0.60	20.72±1.21	10.37±0.59
2 h	+Al/+Al	67.18±1.23	6.53±0.11	15.83±0.99	10.47±0.35

transduction substance ABA might be involved in regulating Al-resistance in soybean.

Roots are fully equipped with all the enzymes and precursors that synthesize ABA. Roots also can take up external ABA. ABA may be a breakdown product of carotenoids, with xanthoxin as an intermediate. FLU can inhibit carotenoids biosyntesis and the absence of ABA in FLU-treated plant seedlings was positively correlated with carotenoid deficiency (Fong et al. 1983; Moore and Smith 1984). In the present study, Al increased the level of endogenous ABA in root apices in dose- and time-dependent manners (Figs. 2, 3). By assaying the monomeric Al concentrations in treatment solutions with pyrocatechol violet method (Kerven et al. 1989), it showed that neither ABA nor fluridone addition affect the monomeric Al concentration in 0.5 mM $CaCl_2$ (pH 4.5) solution (data not shown). However, application of ABA and Flu in the treatment solution can regulate the endogenous ABA accumulation and then Al-resistance in soybean roots (Fig. 1). Exogenous ABA increased ABA accumulation in roots, and then alleviated Al-induced root elongation of soybean (Fig. 1), whereas in contrast, FLU decreased the endogenous ABA content of roots and aggravated Al toxicity of soybean (Fig. 1), suggesting that ABA may play an important role in regulating Al-resistance in soybean. It is supported by the results in barley (Kasai et al. 1993a, b; Matsumoto et al. 1996), maize (Klimashevskii 1983) and the same pant species, soybean (Shen et al. 2004), in which Al increased the endogenous ABA accumulation and exogenous ABA increased Al tolerance. In barley, it was showed that Al treatment increased ABA levels in roots, and either Al or ABA application can increased vacuolar H^+-ATPase activity in roots (Kasai et al. 1993a, b). Furthermore, vanadate (plasma membrane H^+-ATPase inhibitor) treatment was showed to increase both the

Table 2 Effect of Al on the distribution of [^{3}H]-ABA in soybean when [^{3}H]-ABA was fed through roots. 10-days-old seedlings were cultured in 0.5 m*M* $CaCl_2$ solution (pH 4.5) for 12 h in the dark. To loaded [^{3}H]-ABA, soybean roots were subjected to 0.5 m*M* $CaCl_2$ solution (pH 4.5) containing [^{3}H]-ABA with the radiation intensity of 1×10^{-3}mCi ml^{-1} for 5 min. After 5 min loading, soybean roots were subjected to 0.5 m*M* $CaCl_2$ solution (pH 4.5) with or without $AlCl_3$ (30 μ*M*). After 5 min or 2 h treatment in the light, soybean seedlings were harvested and separated to roots, stems and leaves for radioactivity determination. Vertical bars represent standard deviation of the mean (n=3)

Time	Treatment	Radioactivity (dpm%)		
		Root	Stem	Leaf
5 min	-Al	90.75±1.67	2.15±0.61	7.11±1.14
	+Al	82.95±0.72	4.56±0.81	12.49±1.45
2 h	-Al	64.67±1.14	16.17±1.16	19.16±0.44
	+Al	74.31±1.98	3.40±0.24	22.3±2.15

Table 3 Effect of Al on the distribution of [^{3}H]-ABA in soybean when [^{3}H]-ABA was fed through leaves. 10-days-old seedlings were cultured in 0.5 m*M* $CaCl_2$ solution (pH4.5) for 12 h in the dark. For feeding from leaves, a small cut was made by a blade in the top leaf of soybean and 2 μl [^{3}H]-ABA (1×10^{-3}mCi ml^{-1}) was applied repeatedly to the cut by a small brush. At the same time, the soybean roots were cultured in 0.5 m*M* $CaCl_2$ solution (pH 4.5). After 5 min loading, roots were subjected to 0.5 m*M* $CaCl_2$ solution (pH 4.5) with or without $AlCl_3$ (30 μ*M*). After 5 min or 2 h treatment in the light, soybean seedlings were harvested and separated to roots, stems and leaves for radioactivity determination. Vertical bars represent standard deviation of the mean (n=3)

Time	Treatment	Radioactivity (dpm%)		
		Root	Stem	Leaf
5 min	-Al	1.21±0.26	3.97±0.58	94.82±0.53
	+Al	2.06±0.16	4.28±0.38	93.66±0.35
2 h	-Al	2.27±0.47	11.70±0.45	86.03±0.63
	+Al	6.18±0.25	8.77±0.57	85.04±0.32

endogenous ABA accumulation and the vacuolar H^+-ATPase activity in barley roots (Kasai et al. 1994). Thus, Matsumoto et al. (1996) proposed the sequence in barley response to Al stress as follows: Al stress signal is captured by receptor, increase of ABA level, and then activation of vacuolar ATP and PPi-dependent H^+-pumps. Further investigation was lacking to study the relationship of Al stress, ABA signal and activity of vacuolar H^+-pumps. In the study of Shen et al. (2004), Al increased the endogenous ABA accumulation in soybean roots; furthermore, exogenous application of ABA increased the activity of citrate synthase, decreased Al accumulation and enhanced Al-induced citrate efflux from soybean roots. Thus, it was suggested that ABA signal transduction pathway was involved in the regulation of Al-induced efflux of citrate in soybean roots (Shen et al. 2004). Similar experiments have been conducted on wheat and buckwheat, but come to different conclusions. In buckwheat, exposure to 5 μM ABA in the absence of Al caused a significant secretion of oxalate (Ma et al. 2001). In wheat, exogenous ABA application did not affect Al-induced malate efflux from roots (Ryan et al. 2003). Further research is needed to clarify the role of ABA in the Al-induced secretion of organic acid anions.

Stress such as drought and salt stress dramatically activate ABA biosynthesis, and the increase of ABA levels under these abiotic stresses mainly result from increased de novo biosynthesis. The degradation of ABA appears to be suppressed by stress (Sauter et al. 2001 and references therein). It is generally considered that accumulation of ABA in root resulted in specific gene expression and transformation of ABA from roots to shoots, followed by improving stress-resistance of plant (Sauter et al. 2001 and references therein). In the present study, Al treatment increased the level of endogenous ABA in both roots and shoots of soybean (Fig. 4). Differentially, La only increased the ABA content in roots, but not in shoots (Fig. 4). Our isotope experiment further proved that ABA can transport from roots to shoots quickly, vice versa, furthermore, Al stress accelerated the transport rate (data not shown, Tables 1, 2 and 3).We proposed that ABA might be an Al-induced root to shoot transmission signal and involved in regulating Al-resistance of soybean, which was supported by the study in Arabidopsis (Larsen et al. 1996; Sivaguru et al. 2003). Larsen et al. (1996) found that exposure of roots to Al rapidly induced callose formation in the shoot apex and proposed that plant hormones such as ABA could be involved in Al signal transfer. Sivaguru et al. (2003) indicated that Al-induced *WAK1* expression proceeded in roots and shoots without any considerable lag time. Therefore, they suggested that *WAK1* induction in shoots can not be caused by a direct Al effect but may result from Al-induced signals, such as ABA, originating from roots (Sivaguru et al. 2003).

ABA recirculation is a significant component of root-to-shoot signaling process. ABA recirculation has been detected in white lupin, castor beans and maize (Jeschke et al. 1997a, b; Wolf et al. 1990), and play important role under salt stress, phosphate deficiency and ammonium nutrition (Jeschke et al. 1997a, b; Peuke et al. 1994). Liang et al. (1997) demonstrated that root accumulation of ABA resulted from an enhanced ABA biosynthesis and reduction of

ABA catabolism in roots and an increased import of ABA from shoots. In the present study, split-root experiment and [^{3}H]-ABA radioisotope technique was used to explore the transport and distribution of ABA in soybean. In our split-root experiment, we found that ABA content in the no-Al-treated root part was obviously lower than the Al-treated root part in the single soybean seedling (Fig. 5B). By [^{3}H]-ABA radioisotope techniques, it was also found that ABA preferentially distributed in the Al-exposed root part (Tables 2 and 3). Re-distribution of ABA prior to be the part under stress appears to be an important adaptation mechanism. Under drought stress, ABA was found to transport from plant roots to shoots where it elicits stomatal closure (Davies et al. 1994). In response to water deficit, the increased ABA concentrations in stomata are the result of not only synthesis and redistribution of ABA within leaves, but also synthesis and export from roots (Davies and Zhang 1991; Dodd et al. 1996).

Taken together, our results suggested that ABA may play an important role in regulating Al-resistance of soybean, as for how ABA modulated these processes is in progress.

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