Chinese Science Bulletin

© 2008 SCIENCE IN CHINA PRESS
Springer

Aluminium toxicity targets PIN2 in *Arabidopsis* root apices: Effects on PIN2 endocytosis, vesicular recycling, and polar auxin transport

SHEN Hong[1†], HOU NingYan[1], Markus SCHLICHT [2], WAN YingLang[2], Stefano MANCUSO [3] & Frantisek BALUSKA [2]

[1] College of Resources and Environment, South China Agricultural University, Guangzhou 510642, China;
[2] Institute of Cellular and Molecular Botany, Bonn University, Bonn D-53115, Germany;
[3] Electrophysiology Laboratory, Department of Horticulture, University of Florence, Viale delle Idee 30, I–50019 Florence, Italy

The most obvious symptom of Al toxicity is the inhibition of root growth. However, the mechanism of Al-inhibiting root growth remains to be elucidated. In this study, auxin transport and vesicle movement of an auxin-efflux carrier (PIN2) were investigated in *Arabidopsis* roots in response to Al stress. Results indicated that Al inhibited the apical transport of auxin in root tips of *Arabidopsis* significantly. The severe inhibition was localized in the cells of transition zone, where the concentration of auxin was only 34% that of the control. Brefeldin A (BFA), an inhibitor of vesicle transport, induced the dot-like structure of PIN2 vesicle significantly. Al decreased the size of dot-like structure of PIN2 vesicles. Results of real-time RT-PCR and Western-blotting analysis showed that Al increased the transcript level of *PIN2* and the accumulation of PIN2 protein in horizontal direction of plasma membrane, but decreased its distribution in endosomes, suggesting that Al inhibited the transport of PIN2 vesicles from plasma membrane to endosomes. Results of cytoskeleton-depolymering drugs indicated that it was via the pathway of disruption of actin microfilaments that Al inhibited the transport of PIN2 vesicles. Exposed to Al stress, the cells of elongation zone had less Al uptake and less transport frequency of vesicles than cells of transition zone. Taken together, our results suggested that Al inhibited root growth mainly by modulating the transport of PIN2 vesicles between plasma membrane and endosomes, thus blocking auxin transport and root growth.

Al treatment, *Arabidopsis*, auxin transport, cytoskeleton, PIN2

Al toxicity was one of the major limitations to crop growth. Initial effect of Al toxicity is the inhibition of root growth. Generally speaking, root tip has been regarded as the most sensitive site to Al toxicity. Meanwhile, root tip plays an important role in perceiving gravity and Al signalling[1]. Hou et al.[2] and Baluska et al.[3] found that the gravitropism of maize roots was enhanced after the actin microfilament in the cell of root tips was disrupted. These results suggested that the actin microfilaments of cell in root tip was involved in the signalling transduction of gravitropism. Recent studies indicated that the transition zone of root tip was most Al-sensitive. Localized application of Al inhibited the cell growth of transition zone significantly[4−6]. However, it remains unclear how Al inhibits the cell growth of transition zone.

PIN genes are the most important auxin-efflux carriers. *In-situ* visualization indicated that the polar localization of PIN proteins directed the polar auxin transport

Received March 20, 2008; accepted May 21, 2008
doi: 10.1007/s11434-008-0332-3
†Corresponding author (email: hshen@scau.edu.cn)
Supported by the National Natural Science Foundation of China (Grant Nos. 30771294 and 30471040), the International Foundation for Science of Sweden (Grant Nos. C/3042-1,2), and the DAAD (Ref423)

and its asymmetric distribution[7−9]. In *Arabidopsis* and maize, PIN protein vesicles transported between plasma membrane and intercellular compartments rapidly[10]. Light play an important role in mediating PIN2 intracellular distribution[11]. Al decreased the secretion of Golgi, and induced the reorganization of vesicle bunches[12−14]. Up to now, little is known of *PIN*s regulate root growth. In this study, experiments with confocal laser microscope were performed to examine the Al effect on the distribution of PIN2 proteins and auxin transport using *PIN2::GFP* transgenic *Arabidopsis* seedlings. The results will provide evidence for how Al inhibits root growth.

1 Materials and methods

1.1 Materials culture

Transgenic *Arabidopsis* seeds including an auxin efflux carrier (*PIN2::GFP*), an actin microfilaments (GFP-fimbrin actin-binding domain 2, *ABD2::GFP*), and a microtubules (*MAP4::GFP*) material were purchased from *Arabidopsis* Biological Resources Center, Ohio State University, USA and Institute of Cellular and Molecular Botany, Bonn University, Germany. The *Arabidopsis* seeds were sterilized in an eppendorf tube as follows: The seedlings were sterilized firstly by 75% of ethanol for 2 min and submerged in 10% sodium hypochlorite for 3 min followed by a 3-min washing with deionized water. In a clean bench, the sterilized seeds were sown on an agar-solidified nutrient medium containing 1/6 MS solution, pH5.8, 1% sucrose. After sowing, the dishes were placed vertically in a growth chamber. The environmental conditions were at 25℃, 14 h/10 h day/night rhythm. After 6-d growth, the seedlings were used for different treatments. All chemical reagents were purchased from Sigma Company (St. Louis, MO, USA). BFA (brefeldin A), latrunculin B, and oryzalin were dissolved in DMSO or methanol into a stock solution of 10^{-2} mol/L. The stock solution was diluted into working solution when being used. Each treatment had 5 replicates. After treatment, the seedling roots were used for further analysis.

1.2 Root elongation experiments

Under the sterilized conditions, *Arabidopsis* seeds were planted on an agar-solidified MS medium. After 6-d growth, the seedlings of similar size were transferred into 0 or 100 μmol/L $AlCl_3$ for 10 min, and then planted on an agar-solidified MS medium containing 0 or 100 μmol/L $AlCl_3$. The seedling roots were marked with neutral red solution at different positions, and then placed vertically in a growth chamber for another 24 h. Root elongation was measured by a ruler, and root elongation was calculated.

1.3 *In-situ* observation of apical auxin transport

Arabidopsis seedlings were cultured on an agar-solidified MS medium for 6 d, and then transferred to a 0.2 mmol/L $CaCl_2$ solution containing 0, 50 μmol/L BFA, or 100 μmol/L $AlCl_3$ (pH 4.5) for 2 h. After treatment, the seedlings were placed on 0, 50 μmol/L BFA, or 100 μmol/L $AlCl_3$ solidified MS medium. Auxin concentrations in different regions of roots were measured by an IAA-selective microelectrode system. An IAA-selective microelectrode was placed 2 μm from the root surface. The vibrating frequency of sensor was 0.1 Hz between two positions of 10 μm distance. Compared with IAA-reference electrode, the apical IAA content can be measured according to Mancuso et al.[15].

1.4 Confocal laser scanning microscopic observation

Arabidopsis seedlings were cultured on an agar-solidified MS medium for 6 d, and then transferred to a 0.2 mmol/L $CaCl_2$ solution containing 20 μmol/L BFA, 100 μmol/L Al, 20 μmol/L latrunculin, or 10 μmol/L oryzalin for 2 h. After treatment, root apices were placed on the loading glass. A drop of treatment solution was added to the root apices. Then root apices were placed under a confocal laser scanning microscope. During observation, all samples were made under the same conditions of microscope and laser. To avoid long-time observation (over 2 min), a drop of treatment solution was added to root apices during observation. Seedling numbers of observation for each treatment were 10 plants, and typical images were captured. Al accumulation in root apices was measured by indirect fluorescence. Al accumulation in root apices was proportional to the intensity of fluorescence. The detailed method was in accordance with that of Zheng et al.[16]. Confocal laser scanning microscope was Eclipse 800; Nikon, Tokyo, Japan with anargon laser, 488 nm, Bio-Rad, Hercules, CA. Green He, Ne laser was 543 nm and 1.4 Mw, while red diode laser was 638 nm and 5 mW for self-fluorescence detection.

1.5 RNA extraction and real-time RT-PCR

Arabidopsis seedlings were grown in 1/6 MS solution

for 6 d, and then transferred to a 0.2 mmol/L $CaCl_2$ solution containing 0, 20 μmol/L BFA, 100 μmol/L $AlCl_3$, or 20 μmol/L BFA + 100 μmol/L $AlCl_3$ for 2 h. After treatment, the root apices were excised for RNA extraction, and then real-time RT-PCR was performed according to Invitrogen protocols. The sense and antisense primers for *PIN2* gene were 5′-TAT CAA CAC TGC CTA ACA CG-3′ and 5′-GAA GAG ATC ATT GAT GAG GC-3′, respectively. β-tubulin was an internal reference gene (At5g12250). The sense and antisense primers of β-tubulin were 5′-TGG GAA CTC TGC TCA TAT CT-3′ and 5′-GAA AGG AAT GAG GTT CAC TG-3′, respectively.

1.6 Western-blotting analysis

Arabidopsis seedlings were grown in 1/6 MS solution for 6 d, and then transferred to a 0.2 mmol/L $CaCl_2$ solution containing 0, 20 μmol/L BFA, 100 μmol/L $AlCl_3$, or 20 μmol/L BFA + 100 μmol/L $AlCl_3$ for 2 h. After treatment, the root apices were excised. Plasma membrane protein vesicles from root apices were extracted by two- phase-partitioning methods. Plasma membrane proteins were dissolved in SDS-loading buffer, which consisted of 0.125 mmol/L Tris-HCl, pH 7.4, 10% (*w/v*) SDS, 10% (*v/v*) glycerol, 0.2 mol/L DTT, 0.002% (*w/v*) bromocresol blue and 5 mmol/L PMSF. The samples were loaded on a discontinuous SDS-polyacrylamide gel. After SDS-PAGE, the proteins were transferred to a PVDF membrane (0.2 μm), and the hybridization reactions were performed. To identify and quantify the PIN proteins, the blots were incubated with polyclonal antibodies against PINs. The antiserum was diluted to 1∶1000 in TBS-T buffer (1 mmol/L Tris-HCl, pH 8.0, and 0.1% Tween). After washing in TBST, the membrane was incubated at room temperature for 1 h with a diluted secondary antibody. After that, the membrane was detected in BCIP-NPT solution[17].

1.7 Statistic analysis

In water culture experiments we performed 2−4 treatments, each treatment having 5 to 10 replicates. All the data were analyzed statistically by Excel and SAS software.

2 Results

2.1 Effect of Al on root growth

Compared with soybean and rice roots, *Arabidopsis* roots were very fine. To investigate the sensitivity of different root segments to Al stress, *Arabidopsis* seedlings were cultured on an agar-solidified MS medium for 6 d. The seedlings of similar size were selected for root elongation experiments in response to Al stress. Firstly the seedlings were treated in a 0.2 mmol/L $CaCl_2$ solution containing 0 or 100 μmol/L $AlCl_3$ for 10 min, and then placed on an agar-solidified MS medium containing 0 or 100 μmol/L $AlCl_3$ at pH 4.5. The different root segments were marked with neutral red solution. Then the whole dishes were covered by parafilm and placed vertically in a growth chamber for 24 h. Root elongation was measured by a ruler. It was observed that Al decreased root elongation of *Arabidopsis* seedlings significantly. In particular, the 0- to 1-mm root segment was the most Al-sensitive, which was 45% of the control. The other root segments including the 1- to 2-mm, 2- to 3-mm, or 3- to 4-mm root segment were less sensitive to Al stress than the 0- to 1-mm segment. No difference of Al-sensitivity was observed among three root segments (Figure 1). Baluska et al.[18] suggested that for 5 to 7-d *Arabidopsis* seedlings, the apical 0.2- to 0.4-mm zone belonged to transition zone. In the zone, cells were changing their mitotic mode and undergoing a preparatory phase for rapid elongation. Furthermore, cell division in this zone was very frequent. In maize, the transition zone (apical 1- to 2-mm region) was regarded as the most Al-sensitive region[19]. In common bean, the apical 1- to 2-mm region showed much more sensitive to Al stress than other root segments[5].

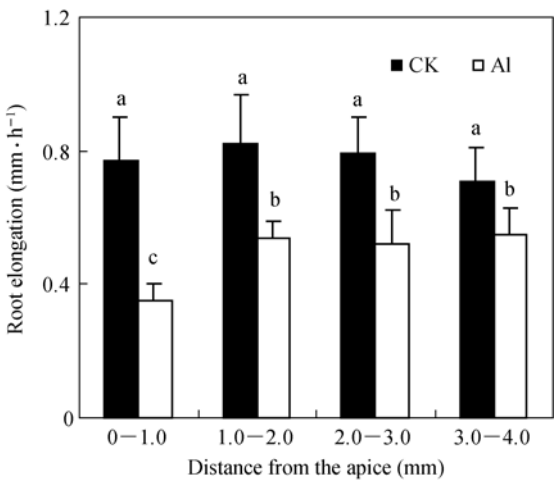

Figure 1 Elongation of different root segment in response to Al stress. The data were means ± SE (n = 10). Different letters indicated significant differences.

2.2 Effect of Al on auxin transport

Auxin plays an important role in regulating root elonga-

tion. Al inhibited the apical 0- to 1-mm zone of *Arabidopsis* seedlings significantly. However, it is unclear whether Al influences the auxin transport. In this study, Al effects on auxin transport were investigated using IAA-selective microelectrode. Results indicated that under normal conditions, auxin concentration in the apical 0.25 mm region was the highest, which was 183 fmol·cm^{-2}·s^{-1}. Treatment with Al or BFA reduced the concentration of auxin greatly (Figure 2). In the region of the apical 0.25 mm, auxin concentration under Al and BFA treatments was 34.3% and 25.1% those of the control (Figure 2). The size of inhibitory effect of Al and BFA was BFA>Al. In the region of the apical 0.5- to 1.5-mm, the decrease of auxin concentration was less than that of the region of the apical 0.1- to 0.4-mm.

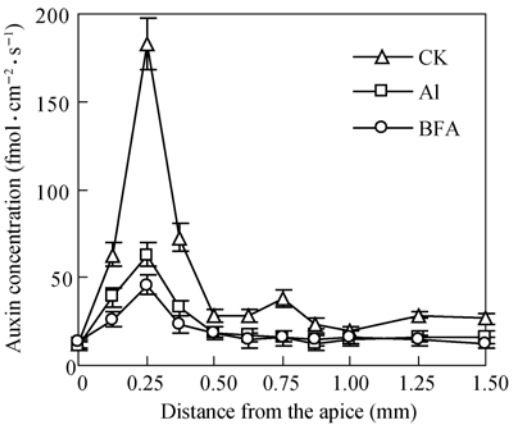

Figure 2 Real-time observation of auxin concentration in root apices of *Arabidopsis* seedlings. *Arabidopsis* roots were treated in 0.2mmol/L $CaCl_2$ solution containing 0, 100 μmol/L $AlCl_3$, 50μmol/L BFA for 2 h, and then transferred to an agar-solidified MS medium. Auxin concentrations in different root segments were measured by IAA-selective microelectrode. The data were Means ± SE (n = 5).

2.3 Effect of Al on PIN2 vesicle trafficking

Brefeldin A (BFA), an inhibitor of vesicle transport had reversible effects on the secretion of Golgi apparatus and endosomes[20,21]. In the cells of root meristem, BFA inhibits exocytosis but allows endocytosis[22]. In this study, treatment with 20 μmol/L BFA induced the formation of dot-like structures in the cells of transition zone significantly as shown in Figure 3(b). Al treatment could not induce the formation of dot-like structures (Figure 3(c)). But Al pretreatment, or Al plus BFA treatment reduced the size of dot-like structures triggered by BFA (Figure 3(d) and (e)). Furthermore, BFA-induced dot-like structure disappeared after 2-h washing with Al solution (Figure 3(f)). Interestingly, the fluoresence intensity of PIN2 in plasma membrane in horizontal direction of

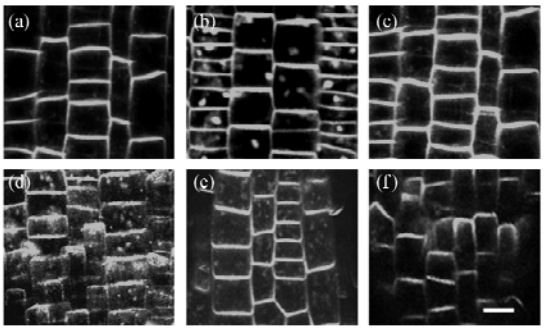

Figure 3 PIN2 vesicle trafficking in the apical cells of arabidopsis seedlings. (a) The control; (b) 20 μmol/L BFA for 2 h; (c) 100 μmol/L Al for 2 h; (d) 100 μmol/L Al for 1 h followed by 20 μmol/L BFA for 2 h; (e) 100 μmol/L Al + 20 μmol/L BFA for 2h; (f) 20 μmol/L BFA for 2 h followed by 100 μmol/L Al for 2 h. Bar indicated 8μm.

cells strengthened in response to Al treatment, suggesting the accumulation of PIN2 vesicles on the horizontal direction of plasma membrane (Figure 3(c)).

2.4 Transcriptional and translational expression of *PIN2* gene

Treatment with Al reduced the size of dot-like structures of PIN2 protein in the cells of transition zone (Figure 3). To explore its regulatory mechanism, the effects of Al and BFA on *PIN2* gene were investigated. Results from Figure 4 indicated that Al increased the transcriptional level of *PIN2* gene, but BFA had no impact on the expression of *PIN2* gene. BFA decreased the accumulation of PIN2 vesicles in plasma membrane, but increased its accumulation in endosomes. In contrast, Al treatment increased the distribution of PIN2 vesicles in plasma membrane, and reduced its accumulation in endosomes (Figure 5). Al influenced both transcriptional and trans-

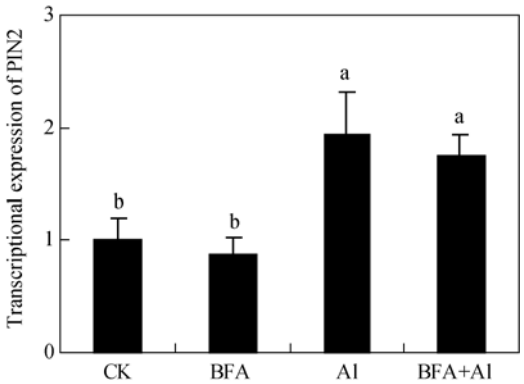

Figure 4 Transcriptional expression of PIN2 gene in the apical cells. Arabidopsis seedlings were cultured in 1/6 MS solution for 6 d, and then transferred to different treatments including the control, 20 μmol/L BFA, 100 μmol/L Al, or 20 μmol/L BFA + 100 μmol/L Al for 2 h. After treatment, the root apices of 1 cm were excised for RNA extraction. Real-time RT-PCR was performed and β-tubulin was as an internal reference. The data were Means ± SE (n = 5).

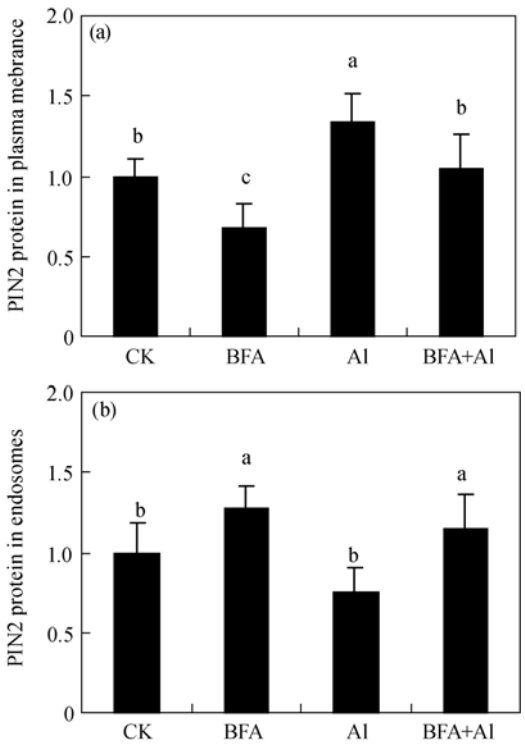

Figure 5 Distribution of PIN2 protein in plasma membrane and endosomes of cells. *Arabidopsis* seedlings were cultured on an agar-solidified MS medium for 6 d, and then transferred to different treatments including the control, 20 μmol/L BFA, 100 μmol/L Al, or 20 μmol/L BFA + 100 μmol/L Al for 2 h. After treatment, root apices of 1 cm were excised, and protein was isolated using two-phase-partitioning method. Western-blotting analysis was performed. The data were Means ± SE (n = 5).

lational expression of *PIN2*, while BFA only influenced its distribution, suggesting that differential mechanisms were associated with the transport of PIN2 vesicles.

2.5 Transport of PIN2 vesicles

Al inhibited the transport of PIN2 vesicle in root cells of transition zone. However, it is unknown how Al blocks the transport of PIN2 vesicle. Using potent cytoskeleton-depolymering drugs, the transport pattern of PIN2 vesicle was investigated in root cells of transition zone. BFA induced the dot-like structures of PIN2 vesicles clearly (Figure 6(b)). Latrunculin, a potential actin-depolymerizing drug, abolished the dot-like structures induced by BFA (Figure 6(c)). Oryzalin, a potent microtubule-depolymerizing drug, had no impacts on the formation of dot-like structure of PIN2 proteins (Figure 6(d)). The results suggested that the transport of PIN2 vesicles in cells was via actin microfilaments rather than microtubules.

2.6 Effect of Al on the cytoskeleton of apical cells

Using indirect immunofluorescent labeling of microtubules and microfilaments, Sivaguru et al.[4] found that treatment with 90 μmol/L $AlCl_3$ for 1 h could induce an obvious variation of actin microfilaments in transition zone cells of maize roots. In this study, the effect of Al on cytoskeleton was investigated in transgenic *Arabidopsis* seedlings including the actin-binding domain 2 of fimbrin gene fused with green fluorescent protein gene (*ABD2::GFP*) and microtubule-associated protein 4 gene fused with green fluorescent protein gene (*MAP4::GFP*). It was observed that treatment with 100 μmol/L $AlCl_3$ for 2 h could result in a reorganization and *PIN2* curving of actin microfilaments in the cells of transition zone of root tips, and some cells were reoriented (Figure 7(b)). In control roots cell microtubules in the transition zone were oriented perpendicularly to the long axis of the root (Figure 3(c)). After Al treatment, the cell microtubules turned extensive; however, the organization of microtubules did not differ from that in control roots (Figure 7(d)).

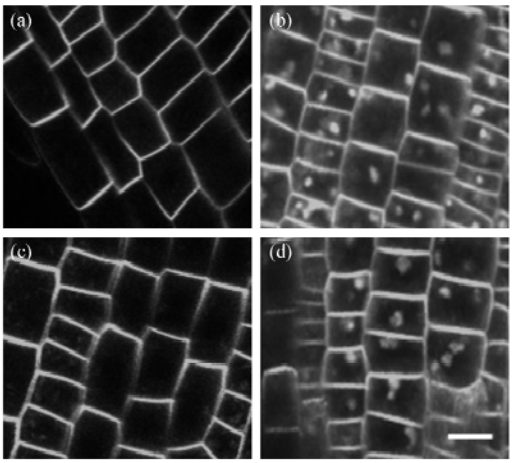

Figure 6 Effect of cytoskeleton-depolymering drugs on the dot-like structures in the cells of transition zone. Arabidopsis seedlings were cultured on an agar-solidified MS medium for 6 d, and then transferred to different treatments including (a) the control; (b) 20 μmol/L BFA; (c) 20 μmol/L BFA + 20 μmol/L latrunculin; (d) 20 μmol/L BFA + 10 μmol/L oryzalin. After treatment, the cells of transition zone of *Arabidopsis* seedlings were observed under a confocal laser scanning microscope. Bar indicated 8 μm.

2.7 Transport of PIN2 vesicles and Al accumulation

In roots of *Arabidopsis* seedlings, transport frequency of PIN2 vesicles in the cells of transition zone and elongation zone showed great difference. Under BFA treatment, the dot-like structures of PIN2 vesicles in the cells of transition zone were much more than those in the cells of elongation zone (Figures 8(b) and (d)). Illes et al.[24] found that the cells of transition zone were more sensi-

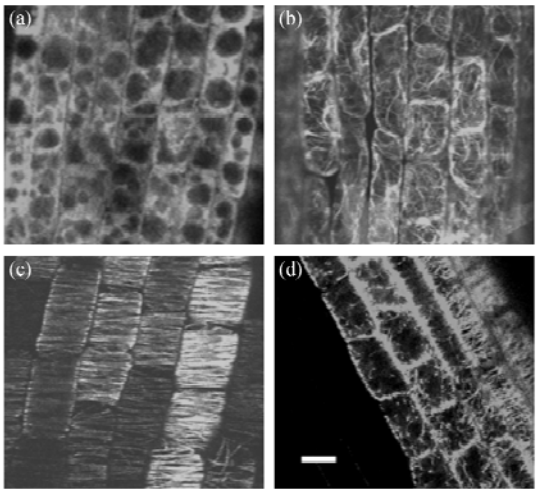

Figure 7 Effect of Al on cytoskeleton of the cells of transition zone. Arabidopsis seedlings were cultured on an agar-solidified MS medium for 6 d, and then transferred to different treatments including (a) the control; (b) 100 μmol/L $AlCl_3$; (c) the control; (d) 100 μmol/L $AlCl_3$ for 2 h. After treatment, the cells of transition zone of Arabidopsis seedlings were observed under a confocal laser scanning microscope. Bar indicated 6μm.

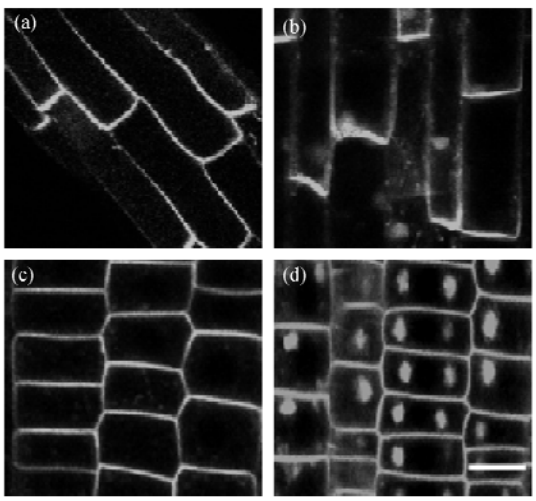

Figure 8 Transport of PIN2 vesicles in the cells of transition zone and elongation zone of *Arabidopsis* roots. (a) and (b) cells in elongation zone; (c) and (d) cells in transition zone; (a) and (c) the control; (b) and (d) 20 μmol/L BFA. *Arabidopsis* seedlings were cultured on an agar-solidified MS medium for 6 d, and then transferred to different treatments for 2 h. After treatment, the apical cells were observed under a confocal laser scanning microscope. Bar indicated 8 μm.

tive to Al stress than those of elongation zone. The accumulation of Al in the cells of transition zone and elongation zone were investigated using morin staining (Figure 8). It was observed that the fluorescence intensity of cells in transition zone was much stronger than that of elongation zone (Figure 9(b) and (d)), suggesting that more Al uptake in the former than in the latter, which was in accordance with the results of Illes et al.[24]. Since morin could not be washed out from root surface

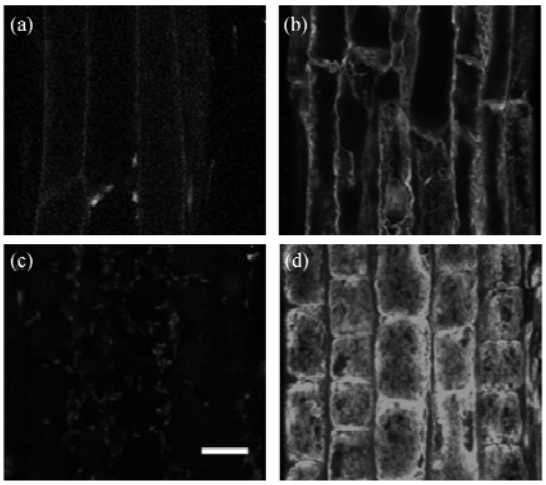

Figure 9 Al accumulation in the cells of transition and elongation zones as detected by the morin fluorescence using confocal laser scanning microscopy. (a) and (b) cells in elongation zone; (c) and (d) cells in transition zone. (a) and (c) the control; (b) and (d) 100 μmol/L Al. *Arabidopsis* seedlings were cultured on an agar-solidified MS medium for 6 d, and then transferred to different treatments for 2 h. After treatment, the apical cells were observed under a confocal laser scanning microscope. Bar indicated 6 μm.

completely, the weak fluorescence intensity was observed even in the control conditions (Figure 9(a) and (c)).

3 Discussion

Baluska et al.[3,18] and Verbelen et al.[25] indicated that the root tip of young *Arabidopsis* seedlings consisted of root cap, apical meristem zone, transition zone, elongation zone and mature zone. In particular, the cells of transition zone were localized mainly in the apical 0.2- to 0.4-mm region. In this region, the cells were growing luxuriantly for cell division and elongation. During the stages, the cells were very sensitive to environmental stress. Results from Figure 1 showed that the apical 0- to 1-mm segment was far more Al-sensitive than other three root segments. The results supported the above view. In accordance with our results, Sivaguru et al.[23] found that the distal part of the transition zone of maize roots were the most sensitive to Al stress. Localized application of Al to the apical 0- to 1-mm region did not influence the growth of elongation zone, but inhibited the growth of transition zone significantly. On maize and common bean, the Al-sensitive region was localized in the apical 1- to 2-mm region. Due to different crop species, the distances between the cells of transition zone and the tip were different. The fact that apical 0- to 1-mm regions of maize and common bean roots were less sensitive to Al stress might be associated with the

complexity of mucilage and Al in root cap, thus preventing the damage of Al on apical cells[4,5].

Auxin is transported from auxin-synthesizing shoot tissues via the phloem toward the root apical meristems, where it is proposed to be unloaded from the central stele into cortical and epidermal cells and then translocated basipetally to the elongation zone[26,27]. Inhibition of basipetal auxin flow has implicated the detrimental effects on root growth and morphology. These include the swelling of root tips through uncontrolled periclinal divisions[8,28]. On the root tips of maize, Al, *n*-1-Naphtylphthalmic acid (NPA) and 2,3,5-triiodobenzoic acid (TIBA) inhibited the transport of auxin from the cells of transition zone to elongation zone. The inhibitory effects of the chemical compounds were NPA>Al> TIBA, and the inhibitory effects were much stronger in Al-sensitive cultivars than in Al-tolerant cultivars[19,29]. Results from Figure 2 indicated that the apical 0.25 mm region accumulated the highest concentration of auxin among the root segments in the control roots. At the region, auxin was much sensitive to Al. Al-induced inhibition of auxin was 66%. Inhibitory effect of Al on auxin transport in the region of apical 0.1- to 0.5-mm was much stronger than in the other root segments. The results were in agreement with the study of Kollmeier et al.[19] and Schlicht et al.[29]. These results supported the view that the cells of transition zone were much sensitive to Al stress due to its high concentration of auxin.

PIN proteins function as a rate-limiting in cellular auxin efflux[30]. As one of the major carriers of auxin efflux, the distribution and cellular orientation of PIN2 mediated the basipetal transport of auxin. In response to gravity, a high concentration of auxin was distributed on the lower side of elongation zone cells, thus mediating the root curvature[31]. Short-term Al treatment may reverse the transport of auxin[32]. Results from real-time RT-PCR and western-blotting analysis indicated that Al raised the transcriptional expression of *PIN2* gene, blocked the trafficking of PIN2 protein from plasma membrane to endosomes (Figures 4 and 5), enhanced the accumulation of PIN2 on plasma membrane, and thus inhibited the transport of auxin (Figure 2). Interestingly, the mechanisms of Al-inhibiting the transport of PIN2 protein were different from those of BFA treatment. Al influencing the dynamics of PIN2 protein was involved in the transcriptional and translational processes, while BFA influenced only the distribution of PIN2 vesicles in root cells. Schwarzerova et al.[14] suggested that Al could induce rapid changes in the microtubular cytoskeleton of tobacco cell lines. After treatment for several hours, Al induced the formation of additional bundles of cortical microtubules. Prolonged exposure of Al resulted in a disorientation of cortical microtubules and a decrease in the cell viability. Figure 7 indicated that Al caused the bending, loosening, and unstabilising of actin microfilaments (Figure 7(b)), but had slight impacts on microtubules, and loosened microtubules slightly (Figure 7(d)). Mathur et al.[33] visualized peroxisomes and cytoskeletal elements, and found that the peroxisome motility was via the pathway of actin microfilaments. Our study confirmed the above results (Figures 6 and 7). Therefore, Al-inhibiting transport of auxin was mainly via the pathway of disrupting actin microfilaments and blocking the transport of PIN2 vesicles between plasma membrane and endosomes. Bao et al.[34] have identified several IAA-responsive genes in *Arabidopsis*. Further studies will be needed to explore the interaction mechanism between Al and IAA-responsive genes in root tips of *Arabidopsis* seedlings.

In rice and wheat, the content of cell wall polysaccharides was associated with the exclusion of aluminum from the root apex[6,35]. Internalization of pectin in cell wall was involved in the Al accumulation in crops, which was highly manifested in the cells of transition zone of *Arabidopsis* seedling[6,24]. In Figure 8, the numbers of dot-like structures induced by BFA were much more in transition zone than in elongation zone, suggesting that the internalization of PIN vesicles were rather frequent in the region. This frequent transport of PIN2 vesicles might be associated with the Al internalization, i.e. Al accumulation.

Taken together, Al mediated the transcriptional and translational expression of PIN2 in the cells of apical transition zone. By disrupting actin microfilaments, Al inhibited the dot-like structures of PIN2 vesicles in the cells of transition zone, and induced the accumulation of PIN2 protein on horizontal plasma membrane. Through the pathway, Al disrupted the basipetal transport of auxin, thus inhibiting the growth of seedling roots. The results obtained in this study will make contribution to the selective breeding of Al-tolerant crops. For example, overexpression of Al-tolerant genes encoding actin microfilaments in crops will be an effective way to avoid the disruption of actin microfilament triggered Al when exposed to Al toxicity, thus guaranteeing the normal transport of auxin and root growth.

1 Ryan P R, Tomaso J M, Kochian L V. Aluminum toxicity in roots: An investigation of spatial sensitivity and the role of the root cap. J Exp Bot, 1993, 44: 437－446 [doi]
2 Hou G, Mohamalawari D R, Blancaflor E B. Enhanced gravitropism of roots with a discrupted cap actin cytoskeleton. Plant Physiol, 2003, 131: 1360－1373[doi]
3 Baluska F, Volkmann D, Barlow P W. Specialized zones of development in roots: View from the cellular level. Plant Physiol, 1996, 112: 3－4
4 Sivaguru M, Horst W J. The distal part of the transition zone is the most aluminum-sensitive apical root zone of *Zeal mays* L. Plant Physiol, 1998, 116: 155－163[doi]
5 Rangel A F, Rao I M, Horst W J. Spatial aluminium sensitivity of root apices of two common bean (*Phaseolus vulgaris* L.) genotypes with contrasting aluminium resistance. J Exp Bot, 2007, 58(14): 3895－3904[doi]
6 Yang J L, Li Y Y, Zhang Y J, et al. Cell wall polysaccharides are specifically involved in the exclusion of aluminum from the rice root apex. Plant Physiol, 2007, 146: 602－611[doi]
7 Galweiler L, Guan C H, Muller A, et al. Regulation of polar auxin transport by *AtPIN1* in *Arabidopsis* vascular tissue. Science, 1998, 282: 2226－2230[doi]
8 Muller A, Guan C, Galweiler L, et al. AtPIN2 defines a locus of *Arabidopsis* for root gravitropism control. EMBO J, 1998, 17: 6903－6911[doi]
9 Wisniewska J, Xu J, Seifertova D, et al. Polar PIN localization directs auxin flow in plants. Science, 2006, 312 (5775): 883[doi]
10 Boutte Y, Crosnier M T, Carraro N, et al. The plasma membrane recycling pathway and cell polarity in plants: Studies on PIN proteins. J Cell Sci, 2006, 119: 1255－1265[doi]
11 Laxmi A, Pan J, Morsy M, et al. Light plays an essential role in intracellular distribution of auxin efflux carrier PIN2 in *Arabidopsis* thaliana. PLoS ONE, 2008, 3(1): 1510－1518[doi]
12 Bennet R J, Breen C M. The aluminum signal: New dimensions to mechanisms of aluminum tolerance. Plant Soil, 1991, 134: 153－166
13 Blancaflor E B, Jones D L, Gilroy S. Alterations in the cytoskeleton accompany aluminum-induced growth inhibition and morphological changes in primary roots of maize. Plant Physiol, 1998, 118: 159－172[doi]
14 Schwarzerova K, Zelenova S, Nick P, et al. Aluminum-induced rapid changes in the microtubular cytoskeleton of tobacco cell lines. Plant Cell Physiol, 2002, 43: 207－216[doi]
15 Mancuso S, Marras A M, Volker M, et al. Noninvasive and continuous recording of auxin fluxes in intact root apex with a carbon-nanotube-modified and self-referencing microelectrode. Anal Biochem, 2005, 341: 344－351[doi]
16 Zheng S J, Yang J L, He Y F, et al. Immobilization of aluminum with phosphorus in roots is associated with high aluminum resistance in buckwheat. Plant Physiol, 2005, 138: 297－303[doi]
17 Shen H, He L F, Sasaki T, et al. Citrate secretion coupled with the modulation of soybean root tip under aluminum stress: Up-regulation of transcription, translation and threonine-oriented phosphorylation of plasma membrane H^+-ATPase. Plant Physiol, 2005, 138: 287－296[doi]
18 Baluska F, Vitha S, Barlow P W, et al. Rearrangements of F-actin arrays in growing cells of intact maize root apex tissues: a major developmental switch occurs in the postmitotic transition region. Euro J Cell Biol, 1997, 72: 113－121
19 Kollmeier M, Felle H H, Horst W J. Genotypical differences in aluminum resistance of maize are expressed in the distal part of the transition zone. Is reduced basipetal auxin flow involved in inhibition of root elongation by aluminum? Plant Physiol, 2000, 122: 945－956[doi]
20 Baluska F, Hlavacka A, Samaj J, et al. F-actin dependent endocytosis of cell wall pectins in meristematic root cells. Insights from brefeldin A-induced compartmentss. Plant Physiol, 2002, 130: 422－431 [doi]
21 Samaj J, Read N D, Volkmann D, et al. The endocytic network in plants. Trend Cell Biol, 2005, 15(8): 425－433[doi]
22 Baluska F, Volkmann D, Menzel D. Plant synapses: Actin-based adhesion domains for cell-to-cell communication. Trend Plant Sci, 2005, 10: 106－111
23 Sivaguru M, Baluska F, Volkman D, et al. Impacts of aluminum on the cytoskeleton of the maize root apex. Short-term effects on the distal part of the transition zone. Plant Physiol, 1999, 119: 1073－1082[doi]
24 Illes P, Schlicht M, Pavlovkin J, et al. Aluminium toxicity in plants: Internalization of aluminum into cells of the transition zone in *Arabidopsis* root apices relates to changes in plasma membrane potential, endosomal behaviour, and nitric oxide production. J Exp Bot, 2006, 57: 4201－4213 [doi]
25 Verbelen J P, De Cnodder T, Le J, et al. The root apex of *Arabidopsis thaliana* consists of four distinct zones of cellular activities: meristematic zone, transition zone, fast elongation zone, and growth terminating zone. Plant Signal Behav, 2006, 1: 296－304
26 Estelle M. Polar auxin transport: new support for an old model. Plant Cell, 1998, 10: 1775－1778[doi]
27 Hasenstein K H, Blancaflor E B, Lee J S. The microtubule cytoskeleton does not integrate auxin transport and gravitropism in maize roots. Physiol Plant, 1999, 105: 729－738[doi]
28 Hasenstein K H, Evans M L. Effects of cations on hormone transport in primary roots of *Zea mays*. Plant Physiol, 1988, 86: 890－894
29 Schlicht M, Miroslav S, Michael J S, et al. Auxin immunolocalization implicates vesicular neurotransmitter-like mode of polar auxin transport in root apices. Plant Sign Behavior, 2006, 1(3): 122－134
30 Petrasek J, Mravec J, Bouchard R, et al. PIN proteins perform a rate-limiting function in cellular auxin efflux. Science, 2006, 312: 914－918 [doi]
31 Abas L, Benjamins R, Malenica N, et al. Intracellular trafficking and proteolysis of the *Arabidopsis* auxin-efflux facilitator PIN2 are involved in root gravitropism. Nat Cell Biol, 2006, 8(3): 249－256[doi]
32 Doncheva S, Amenos M, Poschenrieder C, et al. Root cell patterning: A primary target for aluminium toxicity in maize. J Exp Bot, 2005, 56: 1213－1220[doi]
33 Mathur J, Mathur N, Hulskamp M. Simultaneous visualization of peroxisomes and cytoskeletal elements reveals actin and not microtubule-based peroxisome motility in plants. Plant Physiol, 2002, 128: 1031－1045[doi]
34 Bao F, Hu Y X, Li J Y. Identification of IAA-responsive genes in *Arabidopsis* thaliana using cDNA microarray techniques. Chin Sci Bullet, 2001, 46(23): 1988－1992 [doi]
35 Hossain A Z, Koyama H, Hara T. Growth and cell wall properties of two wheat cultivars differing in their sensitivity to aluminum stress. J Plant Physiol, 2006, 163: 39－47