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Induction of Microsomal Membrane Proteins in Roots of an Aluminum-Resistant Cultivar of *Triticum aestivum* L. under Conditions of Aluminum Stress[1]

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Three-day-old seedlings of an Al-sensitive (Neepawa) and an Al-resistant (PT741) cultivar of *Triticum aestivum* were subjected to Al concentrations ranging from 0 to 100 μM for 72 h. At 25 μM Al, growth of roots was inhibited by 57% in the Al-sensitive cultivar, whereas root growth in the Al-resistant cultivar was unaffected. A concentration of 100 μM Al was required to inhibit root growth of the Al-resistant cultivar by 50% and resulted in almost total inhibition of root growth in the sensitive cultivar. Cytoplasmic and microsomal membrane fractions were isolated from root tips (first 5 mm) and the adjacent 2-cm region of roots of both cultivars. When root cytoplasmic proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, no changes in polypeptide patterns were observed in response to Al stress. Analysis of microsomal membrane proteins revealed a band with an apparent molecular mass of 51 kD, which showed significant accumulation in the resistant cultivar following Al exposure. Two-dimensional gel analysis revealed that this band comprises two polypeptides, each of which is induced by exposure to Al. The response of the 51-kD band to a variety of experimental conditions was characterized to determine whether its pattern of accumulation was consistent with a possible role in Al resistance. Accumulation was significantly greater in root tips when compared to the rest of the root. When seedlings were subjected to Al concentrations ranging from 0 to 150 μM, the proteins were evident at 25 μM and were fully accumulated at 100 μM. Time-course studies from 0 to 96 h indicated that full accumulation of the 51-kD band occurred within 24 h of initiation of Al stress. With subsequent removal of stress, the polypeptides gradually disappeared and were no longer visible after 72 h. When protein synthesis was inhibited by cycloheximide, the 51-kD band disappeared even when seedlings were maintained in Al-containing media. Other metals, including Cu, Zn, and Mn, failed to induce this band, and Cd and Ni resulted in its partial accumulation. These results indicate that synthesis of the 51-kD microsomal membrane proteins is specifically induced and maintained during Al stress in the Al-resistant cultivar, PT741.

Soluble Al in acid soils is a major factor limiting agricultural production on a worldwide basis. Even at low concentrations, Al is known to modify plant growth at the organ, tissue, and cellular levels. Although many of the toxic effects of Al on plant growth and function have been described (Foy et al., 1978; Haug, 1984; Taylor, 1988; Haug and Shi, 1991; Matsumoto, 1991), little is known about the actual mechanisms of resistance to Al. Several exclusion mechanisms have been proposed, including modification of the Al-binding properties of the cell wall; efflux of Al, phosphate, or chelator ligands; selective permeability of the plasma membrane; and alkalization of the rhizosphere (Taylor, 1988, 1991). Internal resistance mechanisms that have been postulated include chelation of Al in the cytosol with organic acids or acidic polypeptides, compartmentation of Al in the vacuole, induction of protein synthesis, synthesis of Al-resistant isozymes, and synthesis of specific Al-binding proteins (Taylor, 1988, 1991). Unfortunately, direct evidence linking the operation of any of these mechanisms with improved resistance to Al is lacking, and experimental work addressing the phenomenon of Al resistance at the molecular level is limited.

One of the first effects of Al toxicity is seen in the form of inhibition of root growth, and the root tip has been identified as the region where stress is first perceived. This is well illustrated by the results of Ryan et al. (1993), who observed that application of Al directly to the root apex inhibited root growth in an Al-sensitive cultivar of *Zea mays*, whereas no effect was observed in an Al-resistant cultivar. When Al was supplied to differentiated regions of the roots, no effect on growth was observed in either cultivar (Ryan et al., 1993). Therefore, the root tip is the tissue of choice for investigating mechanisms of Al resistance. It is likely that resistance is mediated by one or more proteins. These proteins could be membrane proteins that actively export Al, enzymes involved in the synthesis or export of chelator ligands, or enzymes responsible for the synthesis of cellular components (such as cell wall material or membrane components) that have properties that confer Al resistance (Taylor, 1991).

Indirect evidence that Al induces the synthesis of proteins in roots of *Triticum aestivum* (wheat) was presented by Aniol (1984). Working with the same species, Rincon and Gonzales (1991) showed that several high, intermediate, and low mol wt polypeptides are induced in the root tips of an Al-sensitive (Tam 105) and an intermediate cultivar (Bounty 203-A) within 24 h of exposure to Al. Similar studies by Delhaize et al. (1991) using cv Egret (Al sensitive) and cv Carazinho (Al resistant) indicated that the synthesis of most Al-induced polypeptides was common to both sensitive and resistant

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Abbreviations: 2D, two-dimensional; RMP51, 51-kD root microsomal protein.

cultivars. Furthermore, polypeptides unique to resistant cultivars did not co-segregate with the resistant phenotype. Picton et al. (1991) also observed Al-induced proteins in root tips of the Al-sensitive cultivar Warigal and the Al-resistant cultivar Waalt. Al appeared to cause changes in both microsomal and cytoplasmic fractions, but the cytoplasmic fraction appeared to undergo more protein changes. In contrast, Ownby and Hruschka (1991) were unable to find microsomal proteins that were selectively enhanced or induced exclusively in the Al-resistant cultivar (T-84) of *Triticum aestivum*. These studies remain inconclusive in attributing a physiological function to any of these Al-induced proteins.

Because the plasma membrane is likely one of the first targets of Al injury and plays an important role in regulating the entry of Al into the cells (Shi and Haug, 1990), we have concentrated our efforts on the membrane component of roots to identify proteins that could be affected by Al stress. In the present study, we have identified a 51-kD polypeptide (or polypeptides) in microsomal membrane preparations that appears only in the Al-resistant cultivar (PT741) upon exposure to Al. Its dose-dependent accumulation in the presence of Al and its disappearance by subsequent removal of Al stress suggests a possible involvement in Al resistance.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Seeds of an Al-resistant (PT741) and an Al-sensitive (Neepawa) cultivar of wheat (*Triticum aestivum* L.) were surface sterilized in 1% (w/v) sodium hypochlorite for 20 min and germinated for 6 to 7 h in double-distilled H_2O containing 0.005 g L^{-1} of Vitavax to control fungal growth. Seedlings were grown for 3 d on nylon mesh suspended over 10 L of aerated nutrient solution containing (in μM) 2900 NO_3, 300 NH_4, 100 PO_4, 800 K, 1000 Ca, 300 Mg, 101 SO_4, 34 Cl, 60 Na, 10 Fe, 6 B, 2 Mn, 0.15 Cu, 0.5 Zn, 0.1 MO, and 10 EDTA (pH 4.50) in a growth chamber (16 h of light, 20°C, 68% RH and 8 h of darkness, 16°C, 85% RH). For Al exposure, 3-d-old seedlings were transferred to treatment solutions containing (in μM) 1000 Ca, 300 Mg, 300 NH_4, and 2900 NO_3, with Al ranging from 0 to 150 μM supplied as $AlCl_3$ and grown for the required period. For other metal ion treatments, 3-d-old seedlings were transferred to treatment solutions containing (in μM) $CdSO_4 \cdot 8H_2O$ (20), $CuSO_4 \cdot 5H_2O$ (30), $MnCl_2 \cdot 4H_2O$ (200), $NiSO_4 \cdot 6H_2O$ (20), or $ZnSO_4 \cdot 7H_2O$ (150). Taylor et al. (1991) demonstrated that these concentrations of metals reduced growth of *T. aestivum* by approximately 50%. In all experiments solution pH was adjusted to 4.50. After growth in the treatment solution, root tissue was excised and immediately frozen in liquid nitrogen and stored at −80°C for later use. Terminal 5-mm sections of roots were obtained for root tips.

For heat-shock treatment, seedlings were grown in aerated nutrient solution (same as above) for 3 d and transferred to a growth chamber and maintained at 42°C for 4 h. Cold stress was imposed on 3-d-old seedlings by maintaining them at 4°C for 24 h. Inhibition of protein synthesis was achieved by supplying 10 and 30 μg mL^{-1} of cycloheximide to 3-d-old seedlings and maintained for different intervals of time up to 96 h.

Preparation of Microsomal Membranes

All operations for preparation of microsomal membrane fractions were carried out at 4°C. Tissue (1 g) was ground with a mortar and pestle in an ice-cold homogenization medium (1:1 ratio) consisting of 50 mM Mops, 2 μM PMSF, 1 mM DTT, and 1 mM EDTA (pH 6.5). The homogenate was then filtered through two layers of cheesecloth and centrifuged at 20,000*g* for 10 min in a Beckman J2–21 M/E centrifuge with a JA20 rotor. The resulting supernatant was centrifuged again at 120,000*g* for 45 min in a Beckman L8-M ultracentrifuge with an SW 40 swing-out rotor. The supernatant was regarded as the cytoplasmic fraction. The microsomal fraction (pellet) was then resuspended in 50 μL of suspension buffer, consisting of 50 mM Mops, 1 mM EDTA, 1 mM DTT, and 1 μM PMSF (pH 6.5) and thoroughly homogenized with a 5-mL glass homogenizer. Aliquots of microsomal membrane and cytoplasmic fractions were stored at −70°C for further analysis. Protein concentrations were determined by the method of Lowry et al. (1951) with BSA as the standard.

Gel Electrophoresis

SDS-PAGE was performed according to the method of Laemmli (1970) using a Protean II-XI or Mini-Protean II electrophoresis cell (Bio-Rad). Microsomal membrane samples (80–100 μg of protein) were mixed with 50 μL of sample buffer (0.125 M Tris-Cl, 4% [w/v] SDS, 20% [v/v] glycerol, 10% [v/v] 2-mercaptoethanol) and incubated at 70°C for 20 min following the procedure of Fernando et al. (1990). Proteins were electrophoresed at 10 mA in the stacking gel (4% total monomer concentration, 2.7% cross-linking monomer solution) followed by 20 mA in the separating gel (10% total monomer concentration, 2.7% cross-linking monomer solution).

For 2D gel electrophoresis, total microsomal membrane proteins were extracted with phenol and precipitated by ammonium acetate in methanol (Hurkman and Tanaka, 1986). The precipitate was washed two times with 0.1 M ammonium acetate in methanol and twice with acetone. The pellet was dried and solubilized in a urea buffer (9.5 M urea, 2% [w/v] CHAPS, 5% [v/v] 2-mercaptoethanol, 1.6% [v/v] Pharmalyte 5/8, and 0.4% [v/v] Pharmalyte 3/10). IEF gel electrophoresis was performed according to the method of O'Farrel (1975). The gels were prerun at 200 V for 10 min, 300 V for 15 min, and 400 V for 15 min to set up a pH gradient. Samples were loaded immediately to IEF tubes (1 mm i.d.), and the gels were electrophoresed for 10 min at 500 V and for 3 h at 750 V. For second-dimension gel electrophoresis, IEF gels were extruded and loaded onto polyacrylamide gels (thickness 1 mm). Proteins were visualized by silver staining using a procedure modified from that of Morrissey (1981). Gels were incubated in a solution of 45% (v/v) methanol and 12% (v/v) acetic acid for 30 min, 10% (v/v) ethanol and 5% (v/v) acetic acid for 3 × 10 min, 2.5% (v/v) glutaraldehyde for 30 min, water for 3 × 10 s and 3 × 10 min, DTT (5 μg mL^{-1}) for 30 min, and 0.012 M $AgNO_3$ for 30 min. For protein coloration, the gels were washed with water three times and then soaked in a solution of 0.28 M

Na_2CO_3/2 mM formaldehyde until bands appeared. The bands were fixed by placing the gels in 1% (v/v) glacial acetic acid for 10 min. The apparent molecular masses of polypeptides were calculated based on the mobilities of protein standards. Standards (Sigma) consisted of β-galactosidase (116 kD), phosphorylase b (97 kD), BSA (66 kD), fumarase (48.5 kD), carbonic anhydrase (29 kD), β-lactoglobulin (18.4 kD), and α-lactalbumin (14.2 kD).

Densitometer Scanning

After the samples were destained, densitometer tracings of protein lanes were obtained with a Beckman DU spectrophotometer using a softpac program Gel Scan Area. Scanning was performed at a wavelength of 520 nm. The *A* was baseline corrected at a region of the gel that showed no protein bands, and relative quantities of the stained proteins were estimated.

RESULTS

Three-day-old seedlings of an Al-sensitive (Neepawa) and an Al-resistant (PT741) cultivar of *T. aestivum* were grown in media containing varying concentrations of Al (0–100 μM). After 3 d of growth in solutions containing 25 μM Al, the root length increment (final length minus initial length) was reduced by 57% in the sensitive cultivar, a concentration that had no effect on root growth in the resistant cultivar. Root growth was almost completely inhibited at 100 μM in the sensitive cultivar, whereas the decline was significantly less in the resistant cultivar (Fig. 1). Root weight, shoot weight, and shoot length were relatively unaffected by this short-term Al exposure in both cultivars (data not shown). From these results, we selected 100 μM as an appropriate concentration of Al to be used to observe protein differences in *T. aestivum* seedlings.

In our next experiments, two cultivars (Al-sensitive Neepawa and Al-resistant PT741) were grown for 3 d in complete nutrient solution and then subjected to 0 or 100 μM Al for 72 h. When microsomal proteins were isolated from the root tips

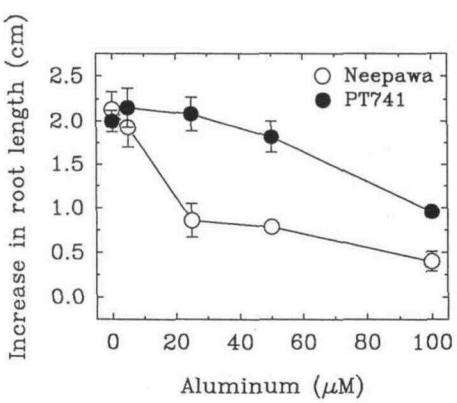

Figure 1. Effect of Al on root elongation in cv Neepawa (sensitive) and cv PT741 (resistant). Plants were grown in nutrient media for 3 d and in treatment solutions containing 0 to 100 μM Al for 96 h. The increase in root length was measured as final root length − initial root length. Values are means ± SE of five replicates.

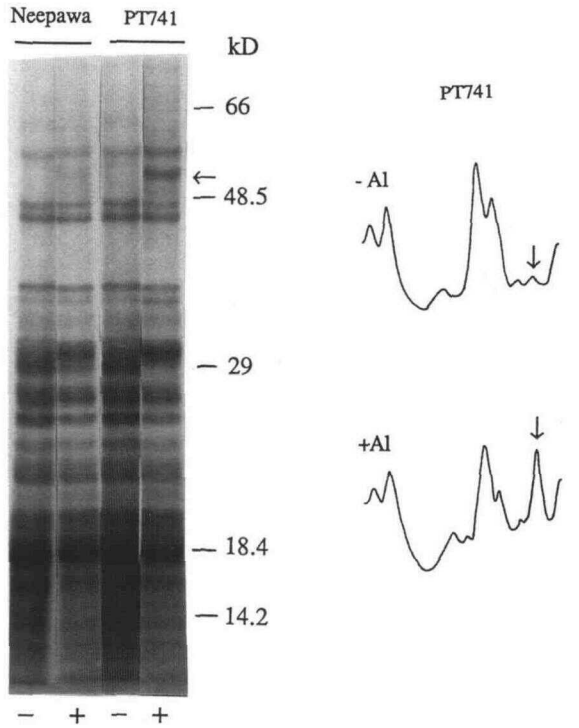

Figure 2. SDS-PAGE analysis of microsomal proteins from roots of cv Neepawa and cv PT741. Three-day-old seedlings were grown in the presence and absence of 100 μM Al for 96 h and microsomal fractions were isolated from 2-cm root tips. Samples were mixed with an equal volume of 2× SDS sample loading buffer and incubated at 70°C for 20 min. Approximately 35 μg of protein were loaded onto each lane. Gels were stained with Coomassie blue. The arrow indicates the relative mobility of RMP51 (51 kD). The molecular mass of marker proteins are indicated on the right in kD. Densitometer scans correspond to PT741 lanes (−Al) and (+Al) from 35- to 63-kD region of the gels. Triplicate samples were prepared and analyzed independently by SDS-PAGE. Similar results were obtained for all independent samples.

(first 2 cm) of treated seedlings and analyzed by SDS-PAGE, a single band of 51 kD (which we shall refer to as RMP51) accumulated in the resistant cultivar when exposed to Al. In the sensitive cultivar, a protein band similar to RMP51 was observed at extremely low levels (Fig. 2). IEF of microsomal membrane proteins from PT741 followed by SDS-PAGE in the second dimension revealed that the RMP51 band comprised at least two polypeptides (Fig. 3B) that accumulate with Al exposure and are observed only at extremely low levels in the control (Fig. 3A). Al treatment also resulted in the suppression of several polypeptides in PT741 (Fig. 3, A and B). A general down-regulation of polypeptides was observed in Neepawa when exposed to Al (Fig. 3, C and D). The SDS-PAGE analysis of cytoplasmic proteins from the supernatant obtained after centrifugation at 120,000*g* revealed no changes in the polypeptide profile as a result of Al stress in either the sensitive or the resistant cultivar (data not shown).

If the polypeptides in the RMP51 band are involved in the Al stress response, we would expect them to accumulate most

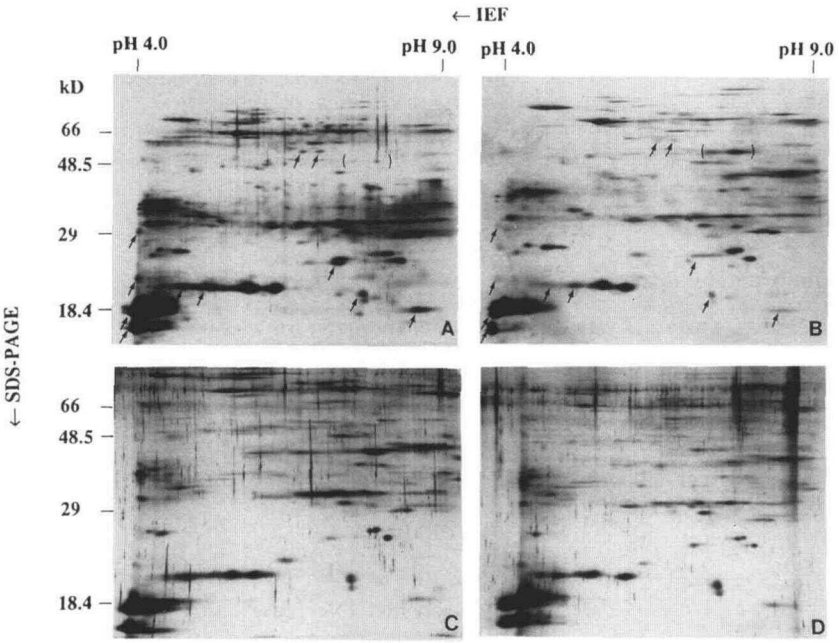

Figure 3. 2D gel analysis of microsomal proteins from roots of cv PT741 without Al (A) and with Al (B) and cv Neepawa without Al (C) and with Al (D). Three-day-old seedlings were grown in the presence and absence of 100 μM Al for 96 h, and microsomal fractions were isolated from the root tips. The proteins were extracted with phenol and precipitated with ammonium acetate in methanol. The pellet was solubilized in a urea buffer before loading onto IEF gels. Separation in the second dimension was performed on 12.5% SDS gels. Gels were silver stained. The molecular masses of proteins are on the left in kD, and the pH scales are indicated at the top. The arrows point to polypeptides that were suppressed by Al treatment. The 51-kD polypeptides are enclosed in parentheses. Triplicate samples were prepared and analyzed independently, and similar results were obtained for all independent samples.

abundantly in tissues experiencing stress. Ryan et al. (1993) investigated the spatial sensitivity of roots of *Z. mays* to Al and found that inhibition of growth occurred only when the terminal 2 to 3 mm of the roots were exposed to Al, implying that Al stress is perceived at the root tip. In our study, when microsomal proteins from different regions of the roots grown in the absence or presence of Al (100 μM) were analyzed, the RMP51 band was more abundant in the root tip (5 mm) compared with the next 2 cm of the root (Fig. 4). Thus, for the remainder of our experiments, microsomal membrane fractions were prepared from 5-mm root tips.

When 3-d-old seedlings of PT741 were subjected to Al treatment ranging from 0 to 150 μM, enhanced accumulation of the RMP51 band was evident at concentrations as low as 25 μM Al. Maximum concentration was observed at 100 μM Al (Fig. 5). Further increases in Al concentration up to 150 μM did not result in any further enhancement. To determine the time course of accumulation, root tips were harvested from seedlings of PT741 after 0, 24, 48, 72, and 96 h of growth with and without Al stress. When plants were grown and maintained in solutions without Al up to 96 h, the RMP51 band was visible at the beginning but disappeared within 72 h. However, in the presence of 100 μM Al, the level of RMP51 was enhanced and maintained throughout 96 h (Fig. 6). The RMP51 band was visible from the onset of Al treatment at a basal level and was completely induced after 24 h (Fig. 6). This led us to determine the short-term kinetics of induction. When plants were grown in Al for 0, 0.5, 1, 2, 6, and 12 h, RMP51 was found at a low basal level from the earliest stages of exposure (data not shown).

When seedlings were exposed to Al (100 μM) for 24 h, the RMP51 band accumulated prominently. When Al was subsequently removed from the growth media, the band gradually disappeared and was no longer visible after 72 h (Fig. 7A). A similar effect was observed when seedlings were subjected to 100 μM Al for 48 or 72 h and later transferred to Al-free media (Fig. 7, B and C). The RMP51 level was maintained as long as Al stress was maintained, but when the stress was removed, the protein band disappeared within 72 h. Inhibition of protein synthesis using cycloheximide (10 and 30 μg mL^{-1}) resulted in gradual disappearance of the

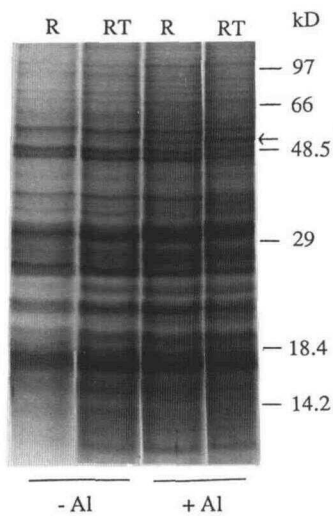

Figure 4. SDS-PAGE analysis of microsomal proteins from 5-mm root tips (RT) and the adjacent 2 cm of the roots (R) of PT741. Three-day-old seedlings were subjected to 0 (−Al) and 100 μM (+Al) Al for 96 h. Microsomal fractions were isolated, and approximately 35 μg of protein were loaded onto each lane. Gels were stained with Coomassie blue. The molecular masses of marker proteins are indicated on the right in kD. Triplicate samples were prepared and analyzed independently by SDS-PAGE. Similar results were obtained for all independent samples.

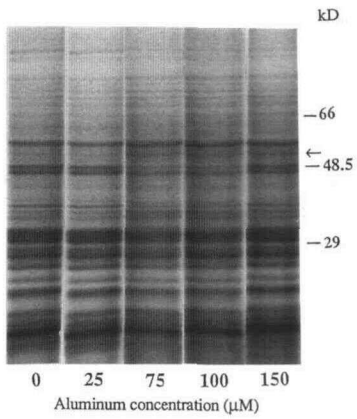

Figure 5. Effect of Al on accumulation of RMP51 in 5-mm root tips of PT741. Three-day-old seedlings were grown for 96 h in media containing 0, 25, 75, 100, and 150 μM Al. Root microsomal fractions were isolated, and 35 μg of protein were loaded per lane. The molecular masses of marker proteins are indicated on the right in kD. Triplicate samples were prepared and analyzed independently by SDS-PAGE. Similar results were obtained for all independent samples.

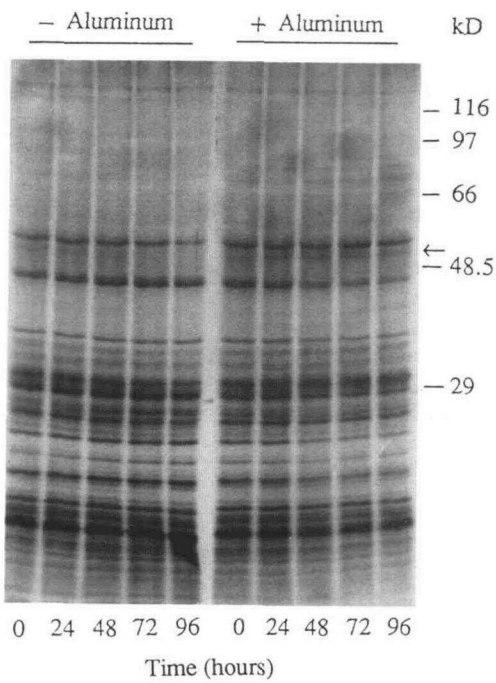

Figure 6. The time course of RMP51 accumulation in PT741. Three-day-old seedlings were transferred to media containing 0 (−Al) and 100 μM (+Al) Al and grown for up to 96 h. Roots were harvested after every 24 h. Microsomal fractions were isolated, and 15 μg of protein were loaded per lane. Gels were silver stained. The molecular masses of marker proteins are indicated on the right in kD. Triplicate samples were prepared and analyzed independently by SDS-PAGE. Similar results were obtained for all independent samples.

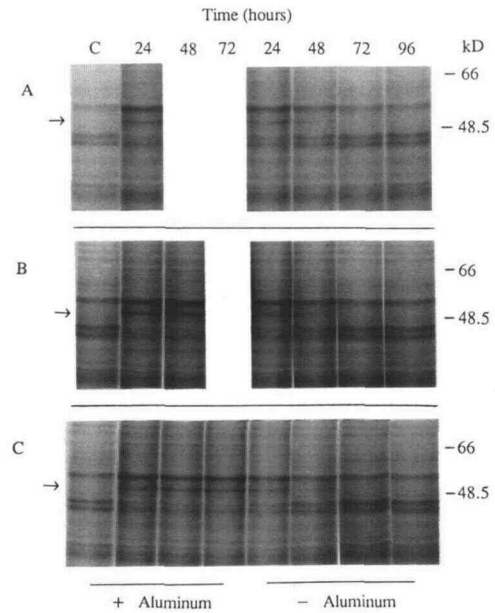

Figure 7. Effect of withdrawal of Al stress on accumulation of RMP51 in PT741. Three-day-old seedlings were exposed to 100 μM Al for 24 (A), 48 (B), or 72 (C) h (+Al) and subsequently grown for another 96 h without Al (−Al). Microsomal fractions were isolated, and 35 μg of protein were loaded per lane. The molecular masses of marker proteins are indicated in kD. Triplicate samples were prepared and analyzed independently by SDS-PAGE. Similar results were obtained for all independent samples. C, Control.

RMP51 band, even when seedlings were maintained on a medium containing Al. Under these conditions, the band was reduced to a basal level by 96 h (data not shown).

To determine whether accumulation of the RMP51 band is a generalized response to metal stress or specifically induced in the presence of Al, various other metals including (in μM) Cd (20), Cu (30), Ni (20), Mn (200), and Zn (150) were tested. Cd and Ni resulted in partial accumulation of the RMP51 band compared with the control. Cd treatment also resulted in the accumulation of a 40.5-kD band and the suppression of 21-, 17-, and 15-kD bands. Other metals had no detectable effect on the protein profile (Fig. 8). When heat shock and cold stress were administered to 3-d-old seedlings, no difference was observed on the RMP51 band compared with the control (data not shown).

DISCUSSION

Previous studies have clearly demonstrated that accumulation of proteins in roots is affected by exposure to Al (Delhaize et al., 1991; Picton et al., 1991; Rincon and Gonzales, 1991), but evidence linking synthesis of specific proteins to expression of Al resistance is lacking. Several recent studies have suggested that the plasma membrane plays a potential role in Al toxicity by regulating Al entry into the cells (Zhang and Taylor, 1989, 1991; Lindberg, 1990; Shi and Haug, 1990). Therefore, it is reasonable to speculate that proteins involved in Al resistance are localized on the plasma membrane. Accordingly, we have concentrated our efforts

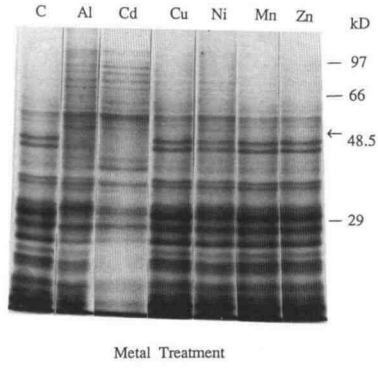

Figure 8. The effect of other metal ions on microsomal proteins. Three-day-old seedlings were transferred to treatment solutions containing toxic concentrations of Al, Cd, Cu, Ni, Mn, and Zn. Concentrations of the metals supplied were (in μM) $CdSO_4 \cdot 8H_2O$ (20), $CuSO_4 \cdot 5H_2O$ (30), $NiSO_4 \cdot 6H_2O$ (20), $MnCl_2 \cdot 4H_2O$ (200), or $ZnSO_4 \cdot 7H_2O$ (150). Triplicate samples were prepared and analyzed independently by SDS-PAGE. Similar results were obtained for all independent samples. C, Control.

on studying differences in the protein profiles of microsomal membranes isolated from root tips. In this study, we observed the accumulation of one or more microsomal membrane proteins (the RMP51 band) in roots of an Al-resistant cultivar (PT741) in response to Al stress. As a group, these polypeptides increase in a time- and dose-dependent fashion and are fully accumulated within 24 h after exposure to Al stress. On 2D gels, the RMP51 band appears to comprise at least two polypeptides, each of which is induced by Al. Further analysis is required to determine whether these represent separate proteins or a single protein in different structural states.

It is interesting that the enhanced level of the RMP51 band could be maintained for more than 72 h only in the presence of Al stress. When we removed the plants from Al-containing nutrient solutions, the level of this band started to decline and disappeared completely by 72 h. Two possibilities could account for the disappearance of the RMP51 band during recovery from Al stress: (a) synthesis of these polypeptides is stimulated by Al, but a regular metabolic degradation of the polypeptides means that continued Al-induced synthesis is required to maintain its level and/or (b) removal of Al stress leads to increased proteolytic degradation of RMP51, leading to declining levels of these polypeptides. To test these hypotheses, we grew seedlings in the presence of Al while inhibiting protein synthesis by cycloheximide. We found a gradual decline in RMP51 levels to basal concentrations within 96 h, indicating that protein synthesis is essential to maintain the enhanced levels in the presence of Al.

Accumulation of the RMP51 band was observed in the Al-sensitive cv Neepawa at extremely low levels. A low basal level was also observed in young seedlings of cv PT741 in the absence of Al stress. It is possible that one or more of the 51-kD polypeptides is developmentally regulated, being present at low basal levels in young seedlings. A second possibility is that the different polypeptides present in the RMP51 band respond differently to Al stress, with perhaps one playing a role in resistance. It is important to recognize, however, that direct evidence that implicates RMP51 in mediating resistance is not yet available. Nonetheless, several observations are consistent with this possibility.

Plants appear to perceive Al stress in the apical regions of the root system (Ryan et al., 1993). Our comparison of RMP51 levels in root tips (5 mm) and the next 2 cm of the root showed that these polypeptides are concentrated in the root-tip region. This suggests that these polypeptides are strategically located to play a role in Al resistance. The finding that protein synthesis is required for accumulation of the RMP51 band is also consistent with our suggestion that one or more of the RMP51 polypeptides may be involved in an Al resistance mechanism. It also appears that these proteins are specifically induced by Al. Environmental stresses arising from other metals (Cu, Mn, Zn), heat shock, and freezing had almost no affect on the accumulation of this band. Partial accumulation was observed in the presence of Cd and Ni.

A number of mechanisms have been suggested that could operate either singly or together to provide Al resistance to plants (Taylor, 1988, 1991). Polypeptides within the RMP51 band might play a role in resistance by mediating either decreased uptake or increased efflux of Al (exclusion of Al). A number of potential exclusion mechanisms have been proposed in the literature, including efflux of chelator ligands, phosphate, or Al (Taylor, 1991). A membrane-bound protein would be required to mediate each of these mechanisms. Novel transport proteins might also be required to maintain normal patterns of ion uptake or perhaps to maintain a relatively high pH in the apoplasm in the face of Al stress. If the polypeptides making up the RMP51 band are associated with endomembranes, they could mediate compartmentation of Al in the vacuole by delivering Al or chelator ligands to the vacuole. They could also play a role in Al resistance as alternative Al-resistant isozymes (Slaski, 1989, 1990).

To the best of our knowledge, this is the first report that specifically deals with membrane protein alterations in response to Al stress. Labeling studies with [^{35}S]Met are currently in progress to further investigate the de novo synthesis of these polypeptides. We are also attempting to discern the segregation of these polypeptides with resistant genotypes. Finally, purification and further characterization of RMP51 polypeptides will be required to confirm a possible role in Al resistance.

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