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Al Inhibits Both Shoot Development and Root Growth in *als3*, an Al-Sensitive Arabidopsis Mutant[1]

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In *als3*, an Al-sensitive Arabidopsis mutant, shoot development and root growth are sensitive to Al. Mutant *als3* seedlings grown in an Al-containing medium exhibit severely inhibited leaf expansion and root growth. In the presence of Al, unexpanded leaves accumulate callose, an indicator of Al damage in roots. The possibility that the inhibition of shoot development in *als3* is due to the hyperaccumulation of Al in this tissue was examined. However, it was found that the levels of Al that accumulated in shoots of *als3* are not different from the wild type. The inhibition of shoot development in *als3* is not a consequence of nonspecific damage to roots, because other metals (e.g. $LaCl_3$ or $CuSO_4$) that strongly inhibit root growth did not block shoot development in *als3* seedlings. Al did not block leaf development in excised *als3* shoots grown in an Al-containing medium, demonstrating that the Al-induced damage in *als3* shoots was dependent on the presence of roots. This suggests that Al inhibition of *als3* shoot development may be a delocalized response to Al-induced stresses in roots following Al exposure.

Al toxicity is a global problem, limiting agricultural productivity in acid soil environments, where the phytotoxic species Al^{3+} predominates (Kochian, 1995; von Uexküll and Mutert, 1995). The biochemical basis for Al toxicity is poorly understood, and the primary sites of Al toxicity have yet to be determined. Initial work in both animal and plant systems has suggested that Al targets several sites on the plasma membrane and cell wall and within the cytoplasm. For example, Jones and Kochian (1995) reported that signal transduction components such as phospholipase C are specifically inhibited following Al exposure.

Other potential targets of Al include ion transporters, in which Al acts to block specifically the uptake of nutrients such as Ca^{+2} (Huang et al., 1992a, 1992b) and K^+ (Gassmann and Schroeder, 1994). Although Al accumulates primarily in the root apoplasm, recent studies indicate that a fraction of the Al enters the root symplasm fairly rapidly (Lazof et al., 1994), where it is thought to interact with many cellular sites (Kochian, 1995), including components of the cytoskeleton such as actin (Grabski and Schindler, 1995). Ultimately, uptake of Al into the root inhibits cell elongation and division, leading to gross changes in root morphology and the inhibition of root growth.

Al toxicity in plants has mostly been examined in relation to its effects on root growth and development. However, one of the long-term effects of growth on acidic Al-toxic soils is the reduction in shoot growth and crop yields (Taylor, 1988). It is thought that the effects of Al on shoot growth are secondary effects due to reductions in nutrient and water uptake by Al-intoxicated roots; Al may block processes necessary for normal development of the shoot. The effects of Al on shoot growth have not been well documented.

We previously reported the isolation and characterization of Arabidopsis mutants with increased sensitivity to Al. These mutants were identified as seedlings with roots that were incapable of growth in mildly inhibitory Al concentrations. Genetic analysis revealed that Al toxicity is genetically complex in Arabidopsis, with at least eight unique loci that, when mutated, confer increased Al sensitivity (Larsen et al., 1996). Al sensitivity could either arise from mutations that cause defects in Al-resistance mechanisms or that enhance mechanisms of Al toxicity. One mutant, *als3*, was subsequently found to be sensitive to Al in both the root and the shoot. In this paper, we examine the inhibition of shoot development in *als3* and attempt to identify the mechanism by which this altered pattern of Al toxicity arises.

MATERIALS AND METHODS

Al-Dependent Inhibition of Seedling Growth

Seedlings were grown on nutrient medium (pH 4.2) containing 0.125% gellan gum (Gell-Gro, ICN). Al was introduced into the gel by equilibration for 2 d with an Al-containing soak solution consisting of a modified nutrient solution (pH 4.2) plus various concentrations of $AlCl_3$ (Larsen et al., 1996). Except where noted, the concentration of $AlCl_3$ in the soak solution was 1.0 mM. The fresh weight of shoots was measured using a microgram balance (model UMT2, Mettler, Highstown, NJ). Al-dependent changes in shoot morphology were assessed following the transfer of 7-d-old seedlings grown on Al-containing medium to PNS (Lincoln et al., 1992) that contained no Al.

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Abbreviations: ICP-MS, inductively-coupled argon plasma MS; PNS, plant-nutrient medium plus Suc.

For exposure of seedlings and excised shoots to Al in liquid culture, five 5-d-old seedlings of wild type and *als3* were transferred to a 24-well plate (Cell Well, Corning, Corning, NY) and incubated for 24 h in a liquid-nutrient medium containing 0, 25, or 50 μM $AlCl_3$. The samples were subsequently transferred to PNS, and the development of shoot inhibition was assessed.

For determination of the effects of other metals on *als3* shoot development, hydroponically grown 5-d-old seedlings were transferred to liquid-nutrient medium or liquid-nutrient medium containing 20 μM $AlCl_3$, 20 μM $LaCl_3$, or 10 μM $CuSO_4$. Following 4 d of exposure, seedlings were transferred to PNS, and shoot inhibition was monitored.

Visualization of Callose Accumulation

Five-day-old, hydroponically grown seedlings were floated in liquid-nutrient medium containing either 0 or 25 μM $AlCl_3$ for 24 h. Seedlings were subsequently fixed in 10% formaldehyde, 5% glacial acetic acid, and 45% ethanol under vacuum for 4 h. To visualize callose accumulation, samples were prepared as reported by Larsen et al. (1996).

Scanning Electron Microscopy

Seedlings were grown for 7 d on gelled-nutrient media equilibrated with either 0 or 1.0 mM $AlCl_3$, and then transferred to PNS. Samples were collected at 1, 4, and 8 d post-transfer, fixed in a solution of 2% glutaraldehyde and 2% paraformaldehyde in 100 mM sodium phosphate buffer (pH 6.8) under vacuum overnight, rinsed twice with 25 mM sodium phosphate buffer, dehydrated using a stepwise series of ethanol substitutions between 25 and 100%, critical-point-dried, sputter-coated, and observed using a scanning electron microscope set at 5 kV (model S-4500, Hitachi, Danbury, CT).

Measurement of Al and Nutrient Accumulation in Shoots

Seedlings were grown hydroponically for 12 d as described by Larsen et al. (1996), except that the pH of the liquid-nutrient medium was adjusted to 5.5. Seedlings were subsequently transferred to liquid-nutrient medium (pH 4.2) containing 15 μM $AlCl_3$. Shoots and cotyledons were harvested at regular intervals after transfer. Following harvest, tissue samples were washed for 15 min twice in ice-cold 0.5 mM citrate (pH 4.2) to remove surface Al. Samples were dried overnight in a 90°C oven and weighed using a microgram balance (Mettler). Dried tissue samples were ashed in 50 μL of hot concentrated nitric acid, resuspended in 2 mL of 1.0% nitric acid, and analyzed using an inductively coupled plasma emission spectrometer (Sciex-Elan 5000, Perkin-Elmer).

To measure the levels of nutrients accumulated in shoots, 12-d-old seedlings were transferred to 15 μM $AlCl_3$ for 3 d. Subsequently, cotyledons were removed and shoots were washed in deionized water for 5 min to remove surface contamination. Samples were prepared as for measurement of Al accumulation. Nutrient concentrations were determined using a trace analyzer emissions spectrometer (model ICAP 61E, Thermo, San Jose, CA).

All equipment used for the analysis of samples was soaked in 20% HCl prior to use to minimize Al contamination.

Genetic Analysis

For mapping purposes *als3* mutants (male parent) were crossed to the Wassilewskija ecotype (Ws-0; female parent). Chromosome location was determined by the identification of microsatellite markers (Bell and Ecker, 1994) and cleaved amplified polymorphic sequences (Konieczny and Ausubel, 1993) that cosegregate with the Al-sensitive trait in F_2 progeny. Map distances were determined using the Mapmaker II program (Lander et al., 1987).

RESULTS

Al-Dependent Inhibition of Root Growth in *als3*

als3 was identified initially as an Arabidopsis mutant in which root growth was more sensitive to Al than the wild type. Some of the physiological and genetic characteristics of *als3* were described in a survey paper on *als* mutants (Larsen et al., 1996). Further genetic analysis of *als3* has shown that the mutation maps to the bottom of chromosome 5 between the microsatellite marker nga129 (10.5 cM, $n = 37$) and the cleaved amplified polymorphic sequences marker CRA1 (13.3 cM, $n = 30$). Further physiological characterization of this mutant indicated that the inhibitory effects of Al were not confined to the root, but also affected shoot development.

When *als3* was grown on gelled-nutrient medium equilibrated with a range of $AlCl_3$ concentrations, root growth was severely inhibited at concentrations of Al that had little effect on wild type (Fig. 1); at concentrations as low as 0.25 mM $AlCl_3$ growth of *als3* roots was reduced. At 1.0 mM $AlCl_3$, the concentration of Al used to screen for *als* mutants, the morphology of *als3* roots was dramatically different from those of the wild type (Fig. 2). After 7 d at this $AlCl_3$ concentration growth of wild-type roots was only moderately inhibited; there was no evidence of Al-dependent alterations in root morphology (Fig. 2, A and B), and there was normal initiation of lateral roots along the length of the primary root. In contrast, Al-treated roots of *als3* were severely stunted (Fig. 2D).

Unlike wild-type roots, which grew moderately well at this concentration of $AlCl_3$, *als3* roots were initially capable of minimal growth, but then growth was completely inhibited. Al-inhibited *als3* roots developed a swollen, club-shaped apex compared with the tapered apex of Al-treated wild-type roots. In addition, root hairs proliferated close to the apex in Al-treated *als3* roots, in contrast to wild-type roots, in which the zone of root hair differentiation started several millimeters behind the root tip. Finally, Al-treated roots of *als3* did not initiate lateral roots, but did produce secondary roots from the base of the hypocotyl. Normally, wild-type Arabidopsis seedlings recover rapidly when transferred to medium without added Al, but the growth

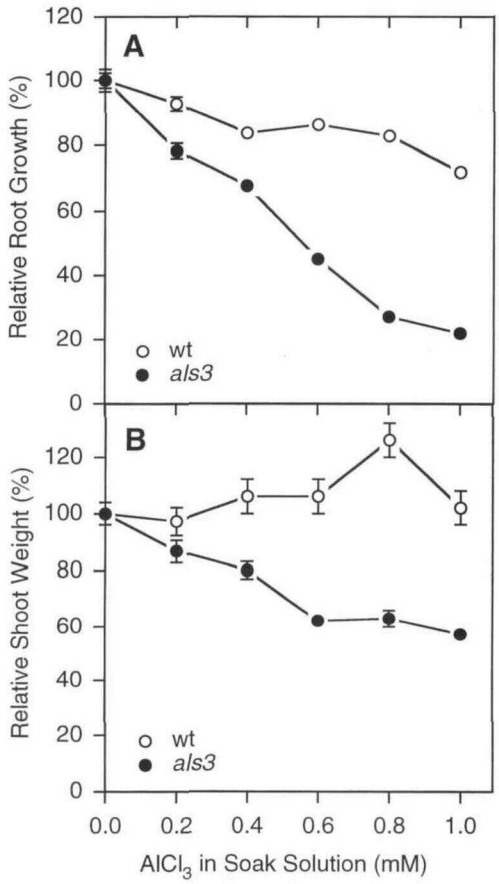

Figure 1. Growth of Arabidopsis seedlings in Al-containing gel media. Seedlings were grown for 7 d on gelled-nutrient medium equilibrated with varying concentrations of $AlCl_3$, after which root length and shoot weight were determined. Relative root (A) and shoot (B) growth are expressed as a percentage of root or shoot growth in the absence of added $AlCl_3$. Error bars represent SE ($n = 20$).

of primary roots in *als3* was irreversibly inhibited. (Even at higher concentrations of Al, when root growth was inhibited by 80–90%, roots of wild-type seedlings were able to recover.) When grown in the absence of Al, roots of *als3* were indistinguishable from those of the wild type (Fig. 2, A and C).

Al-Dependent Inhibition of Shoot Growth in *als3*

In addition to the inhibition of root growth, shoot development in *als3* was also affected following growth in an Al-containing medium. The accumulation of fresh weight in the *als3* shoot was reduced with increasing Al concentration after 7 d of growth (Figs. 1B and 2). Growth of *als3* shoots on medium equilibrated with 1.0 mM $AlCl_3$ was inhibited almost 40%, whereas wild-type shoots were unaffected. At this stage of seedling development, the shoot consisted largely of the hypocotyl and cotyledons. The first true leaves were very small and contributed little to the overall weight of the shoot. Since hypocotyl length in *als3* was comparable to that of wild type following exposure to Al, most of the growth inhibition in *als3* shoots at this stage

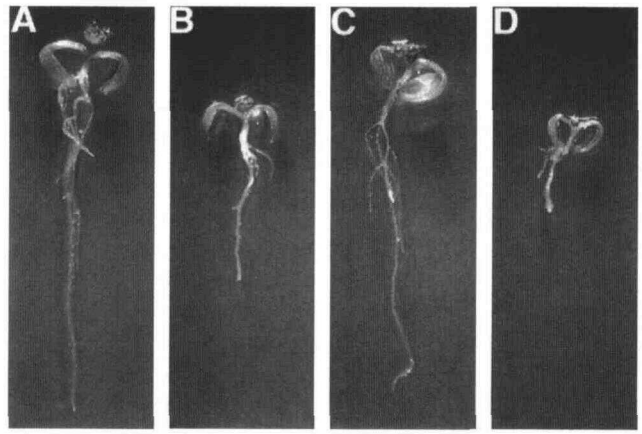

Figure 2. Growth of wild-type and *als3* roots following exposure to Al. Seedlings were grown on a gelled-nutrient medium equilibrated with 0 or 1.0 mM $AlCl_3$ for 7 d. A, Wild type, no Al; B, wild type, 1.0 mM $alCl_3$; C, *als3*, no Al; D, *als3*, 1.0 mM $AlCl_3$.

was related to cotyledon expansion. Without added Al, shoots of *als3* were comparable in fresh weight to wild-type shoots. Inhibition of shoot growth was not simply a threshold effect, which would also have occurred in the wild type at higher Al concentrations; shoot inhibition was not observed in wild type even at Al concentrations that inhibited root growth by 80 to 90% (data not shown).

Further differences between Al-treated seedlings of wild type and *als3* were observed following transfer to a non-Al-containing medium. Leaf expansion was blocked for several days after the transfer (Fig. 3). Examination of the first true leaves of both wild type and *als3* using scanning electron microscopy revealed dramatic alterations in leaf

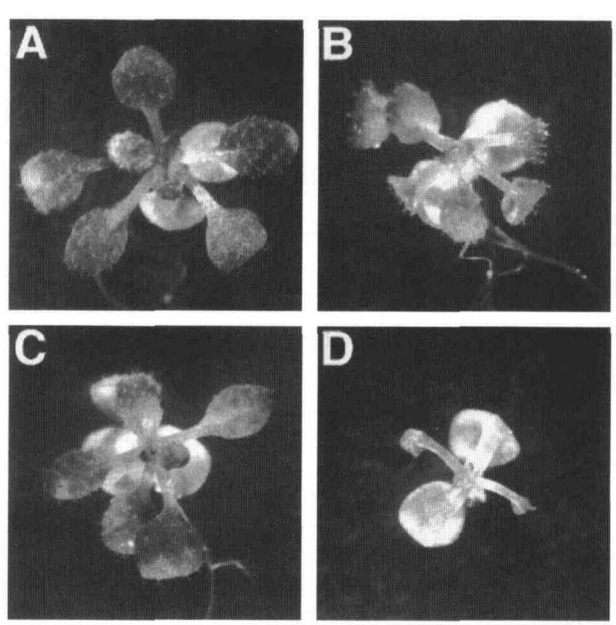

Figure 3. Growth of wild-type and *als3* shoots following exposure to Al. Seedlings were grown on a gelled-nutrient medium equilibrated with either 0 or 1.0 mM $AlCl_3$ for 7 d, and subsequently transferred to PNS for 8 d. A, Wild type, no Al; B, wild type, 1.0 mM $alCl_3$; C, *als3*, no Al; D, *als3*, 1.0 mM $AlCl_3$.

morphology in *als3* (Fig. 4). In wild-type seedlings Al had no effect on leaf initiation or expansion. In contrast, 1 d following removal from the Al-containing medium, the first true leaves of *als3* were severely stunted and very few trichomes were present (Fig. 4B). These leaves continued to develop abnormally, with the petiole and leaf blade elongating, but with little or no expansion in the leaf blade (Fig. 3D). In addition, the surface of the Al-treated leaf of *als3* was rough, with irregularly shaped epidermal cells compared with Al-treated wild-type leaves (data not shown).

Leaves on *als3* that developed after transfer to the non-Al-containing medium also had altered morphology. These leaf primordia did not expand by 4 d after transfer and were a disorganized cluster of small leaf pegs at the shoot apex (Fig. 4D). In contrast, leaf primordia on wild-type seedlings expanded normally (Fig. 4C). By 8 d after transfer, blades of the developed leaf primordia of *als3* had begun to expand, but petiole development was not observed (Fig. 4F). A second shoot apex, complete with a developing rosette of leaves, consistently appeared in Al-treated *als3* seedlings by this time. After further growth *als3* appeared quite normal except that it had an increased number of rosette leaves and inflorescences that often were fused (data not shown); these abnormalities were not observed when *als3* was grown without Al.

Callose Accumulation in Shoots of *als3*

Callose deposition has been used as an indicator of Al-induced stress in roots because callose accumulates to high levels in root tips following exposure to toxic levels of Al (Wissemeier et al., 1987; Llugany et al., 1994). Even though roots of *als3* are more sensitive to Al than those of the wild type, they did not accumulate higher levels of callose when exposed to inhibitory levels of Al (Larsen et al., 1996). Seedlings were exposed to 0 or 25 μM $AlCl_3$ in liquid-nutrient media for 24 h, then examined for callose accumulation. When grown in the absence of Al, neither wild-type nor *als3* shoots accumulated callose in the shoot apex or leaf primordia (Fig. 5, A and C); this was also the case for wild-type shoots exposed to Al (Fig. 5B). In contrast, when grown in the presence of Al, leaf primordia in *als3* were highly fluorescent, indicating the accumulation of significant levels of callose (Fig. 5D).

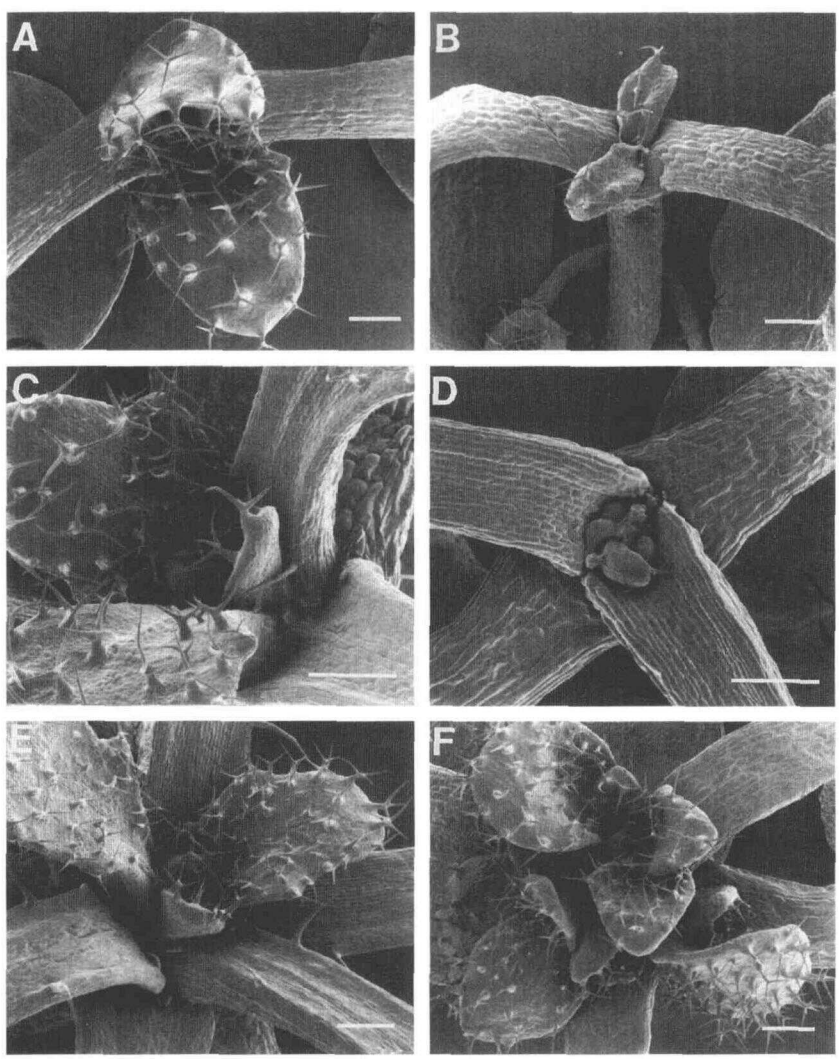

Figure 4. Scanning electron micrographs demonstrating the Al inhibition of leaf expansion in *als3*. Seedlings were grown on a gelled-nutrient medium equilibrated with 1.0 mM $AlCl_3$ for 7 d, and subsequently transferred to PNS. Bars = 300 μm. A, Wild type, 1 d after removal from Al-containing media; B, *als3*, 1 d after removal from Al-containing media; C, wild type, 4 d after removal; D, *als3*, 4 d after removal; E, wild type, 8 d after removal; and F, *als3*, 8 d after removal.

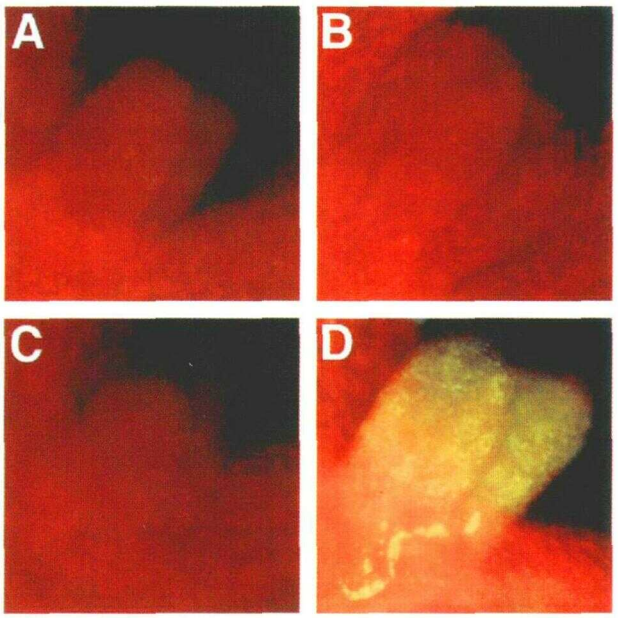

Figure 5. Callose accumulation in developing leaf primordia of *als3* following exposure to Al. Five-day-old, hydroponically grown seedlings were exposed to 0 or 25 μM $AlCl_3$ for 24 h. Seedlings were fixed, stained with 0.1% aniline blue (pH 9.0), and observed using epifluorescence microscopy. A, Wild-type leaf primordia, no Al; B, wild-type leaf primordia, 1.0 mM $alCl_3$; C, *als3* leaf primordia, no Al; D, *als3* leaf primordia, 1.0 mM $AlCl_3$.

Callose fluorescence was observed only in the leaf primordia and was not observed generally in the shoot or cotyledons.

Measurement of Al Accumulation in Shoots

Although we demonstrated in an earlier study that *als3* did not accumulate high levels of Al in root tips when exposed to Al-containing media (Larsen et al., 1996), we wanted to determine whether increased Al sensitivity in shoots of *als3* was due to hyperaccumulation of Al in shoots. The levels of accumulated Al in the shoots of wild type and *als3* were compared following growth in moderate concentrations of Al. Twelve-day-old plants of wild type and *als3* were exposed to 15 μM $AlCl_3$, and cotyledon and shoot samples were harvested at various times. ICP-MS analysis showed that both wild-type and *als3* shoots and cotyledons accumulated similar levels of Al over 2 d of exposure to Al (Fig. 6, A and B). Al concentrations increased through 24 h after transfer, with rates leveling off by 48 h. Since Al uptake rates for cotyledons and shoots of wild type and *als3* were similar, this suggested that Al-sensitivity in *als3* shoots does not arise from increased accumulation of Al.

Measurement of Nutrient Accumulation in Shoots

To determine if the Al-induced inhibition of leaf expansion in *als3* results from nutrient deficiency in the shoot caused by exposure to Al, concentrations of nutrients in shoots of wild type and *als3* were measured following 3 d of exposure to 15 μM $AlCl_3$. Exposure to Al had little effect on the accumulation of macronutrients (K, P, Ca, and Mg) in shoots of either wild type or *als3* (Table I). A comparison of wild type with *als3* revealed a modest increase in P accumulation in *als3* shoots that was independent of Al exposure, but the relevance of this was not clear.

With regard to micronutrients, levels of Cu and Zn were comparable for wild type and *als3*, both in the presence and absence of Al. In contrast, in the absence of Al, levels of both B and Mn were lower in *als3* shoots compared with wild type. Upon exposure to Al, B levels in both wild type and *als3* declined, indicating that B deficiency may be a significant factor for long-term growth in an Al-toxic environment. Al exposure resulted in reductions in Mn levels only in *als3* shoots, leading to about a 50% decrease compared with wild type. The reduced levels of B or Mn are not thought to be responsible for the Al-dependent inhibition of shoot expansion in *als3*, since the levels reported are not considered to be deficient.

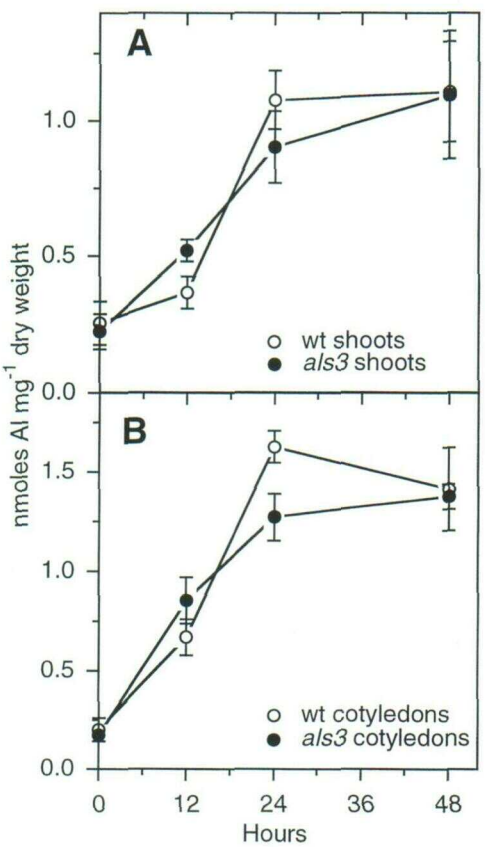

Figure 6. Al accumulation in shoots following growth in Al-containing medium. Twelve-day-old, hydroponically grown seedlings were exposed to 15 μM $AlCl_3$ for various lengths of time, and shoots (A) and cotyledons (B) were collected for measurement of accumulated Al. Samples were washed in 0.5 mM citrate (pH 4.2) to remove surface Al. Dried tissue was ashed in concentrated HNO_3 and analyzed using ICP-MS. Samples consisted of 10 seedlings each. Error bars represent SE (n = 6).

Table I. *Nutrient concentrations in Arabidopsis shoots*

Values are ±SE (n = 6).

Treatment	Nutrient Concentration							
	K	P	Ca	Mg	Mn	Cu	B	Zn
				$\mu mol\ g^{-1}$ *dry wt*				
Wild type −Al	1425.1 ± 20.7	231.4 ± 4.3	288.4 ± 9.0	211.6 ± 3.0	1.96 ± 0.11	0.25 ± 0.01	2.09 ± 0.03	1.25 ± 0.03
Wild type +Al	1477.0 ± 19.8	237.4 ± 4.4	281.2 ± 4.2	231.2 ± 2.6	1.90 ± 0.08	0.24 ± 0.06	1.37 ± 0.02	1.22 ± 0.03
als3 −Al	1399.5 ± 27.8	286.5 ± 4.7	299.6 ± 9.8	211.1 ± 6.6	1.39 ± 0.09	0.22 ± 0.00	1.62 ± 0.08	1.45 ± 0.05
als3 +Al	1436.6 ± 25.1	271.4 ± 2.1	295.2 ± 8.1	255.6 ± 5.5	0.89 ± 0.02	0.25 ± 0.01	1.20 ± 0.04	1.35 ± 0.02

Relationship of Inhibition of Shoot Expansion to Root Damage

We wanted to determine whether the effects of Al on shoot development in *als3* seedlings were the result of increased Al sensitivity or of delocalized expression in shoots of stress signals generated in roots. To test if the inhibition of shoot development in *als3* results from a general stress response, seedlings were grown in the presence of root-growth-inhibiting concentrations of other metal ions, and the effect on shoot growth was monitored. Wild-type and *als3* seedlings were grown in liquid-nutrient medium and after 5 d were exposed to levels of $AlCl_3$, $LaCl_3$, or $CuSO_4$ that caused greater than 80% inhibition of root growth (Table II). Following 4 d of exposure, seedlings were rescued on PNS medium and subsequent shoot development was monitored (Table II). Only $AlCl_3$ inhibited shoot development in *als3* (Fig. 7). Exposure to root-growth-inhibitory levels of $LaCl_3$ or $CuSO_4$ had no effect on the growth of *als3* shoots, indicating that the inhibition of *als3* shoots was not related to general root damage caused by metal ion stress.

The possibility that shoot inhibition resulted from an Al-dependent stress response occurring at the root was also explored by determining whether shoot development was inhibited by Al in excised *als3* shoots. Intact seedlings and excised shoots of wild type and *als3* were exposed to 0 or 50 μM $AlCl_3$ for 24 h and subsequently transferred to PNS medium for monitoring shoot development. By 4 d after transfer, wild-type leaves from both intact seedlings and excised shoots expanded normally (Table III). As expected, root growth and leaf expansion in intact *als3* seedlings treated with Al were severely inhibited. In contrast, excised *als3* shoots developed normally, with rates of leaf expansion comparable to control *als3* seedlings. The dependence of shoot inhibition in *als3* on the presence of roots indicates that the inhibition of leaf expansion in *als3* may be a root-mediated phenotype.

DISCUSSION

Aside from its more extensively documented effects on root growth, Al also has an impact on shoot growth and crop yields (for example, see Taylor [1988]). Some of the toxic effects of Al on shoot growth mimic nutrient deficiencies and are thought to be secondary effects from the inhibition of root growth (Foy, 1984). More direct effects of Al on shoot growth and development are less well documented. The Al-sensitive Arabidopsis mutant *als3* was selected because its primary root growth was more sensitive to Al than was that of the wild type. Further characterization has shown that lateral root initiation and leaf development and expansion in the shoot apex are also sensitive to Al in *als3*. Surprisingly, Al inhibition in the growth of *als3* shoots is correlated with the accumulation of callose, a good indicator of Al toxicity in roots.

The heightened Al sensitivity in *als3* roots does not appear to involve greater uptake of Al in mutant root tips. By various criteria, such as staining with Al-indicator dyes, we know that *als3* does not take up more Al in root tips than does the wild type (Larsen et al., 1996). Furthermore, *als3* shoots do not appear to hyperaccumulate Al; Al content in shoots of wild-type and *als3* seedlings exposed to Al are comparable, as determined by ICP-MS. This is perhaps not surprising, since the Al-induced phenotype of *als3* has not been observed in wild-type Arabidopsis, even at concentrations of Al that are completely inhibitory for wild-type root growth. It is possible that higher levels of Al accumulate locally in a few cells of the *als3* shoot apex, although callose accumulation is not highly localized but occurs generally in expanding leaves of the shoot apex. However, the pattern of callose accumulation might be more widespread than the cells that are primarily affected by Al.

A possible explanation for the shoot phenotype in *als3* is that Al reduces nutrient supplies to the shoot because of reduced nutrient uptake rates in roots (Foy, 1984). This also apparently is not the case, since nutrient levels, with the

Table II. *Root and shoot inhibition after exposure to various metals for 4 d*

Values are ±SE (n = 6).

Treatment	% Root Growth				% with Rosette			
	Control	$AlCl_3$	$LaCl_3$	$CuSO_4$	Control	$AlCl_3$	$LaCl_3$	$CuSO_4$
		20 μM		*10 μM*		*20 μM*		*10 μM*
Wild type	100 ± 2.3	30.9 ± 2.5	15.2 ± 3.1	6.1 ± 2.8	100	100	100	100
als3	100 ± 2.6	7.8 ± 2.8	11.1 ± 1.8	4.1 ± 2.4	100	5	100	100

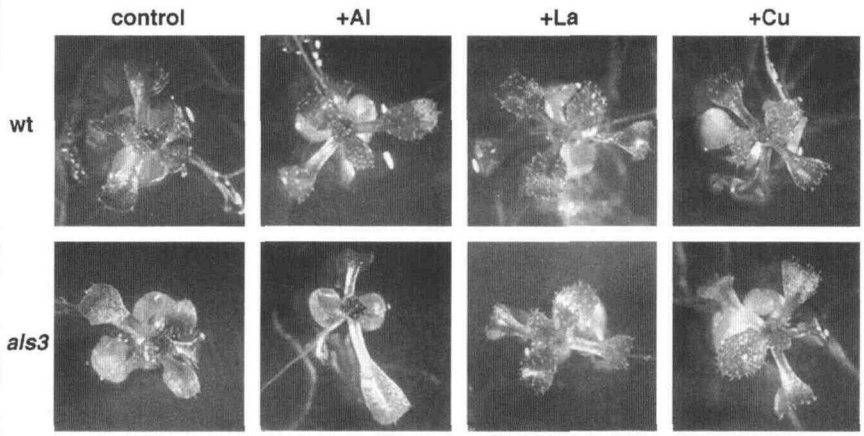

Figure 7. Dependence of inhibition of leaf expansion on exposure to Al. Five-day-old, hydroponically grown seedlings were transferred to either liquid-nutrient medium (control) or liquid-nutrient medium supplemented with 20 μM $AlCl_3$, 20 μM $LaCl_3$, or 10 μM $CuSO_4$. Following 4 d of exposure, seedlings were transferred to PNS medium and the development of shoot inhibition was observed. wt, Wild type.

exception of Mn, are comparable to those of wild-type shoots following Al exposure. Although Mn levels are significantly lower in *als3* compared with wild type, these levels do not represent a deficient state, suggesting that reduced Mn levels are a symptom and not a cause of Al toxicity in *als3* shoots.

The most appealing explanation for the Al-dependent inhibition of leaf enlargement in *als3* centers around an altered interaction between the root and shoot. This conclusion is primarily based on the observation that the appearance of Al toxicity in *als3* shoots requires the presence of the root. One possible scenario for the shoot effect is that Al toxicity in the root results in the transport of some growth-inhibiting substance to the shoot, and the mutant might overproduce or be hypersensitive to that substance in the shoot. Alternatively, Al toxicity in *als3* roots may inhibit the production, translocation, or action of a substance that is necessary for shoot development.

Another possibility is that the response of *als3* shoots to Al may represent a delocalized stress response normally confined to wild-type roots. It has been argued that Al elicits a primary response that is dependent on interactions of Al with sites of toxicity, followed by a secondary, more general stress response. The general stress response includes symptoms such as accumulation of callose and the induction of several genes that apparently have no primary role in either Al tolerance or resistance. For example, the *wali* (wheat aluminum-induced) genes, which are differentially expressed in wheat roots exposed for an extended period to Al, represent a wide range of stress-related genes with no obvious role in Al toxicity (Snowden and Gardner, 1993; Richards et al., 1994; Snowden et al., 1995). These include genes for a metallothionein, a Phe ammonia-lyase, and a Bowman-Birk proteinase inhibitor.

Genes identified in other studies based on their Al-inducibility include *TAl-18*, a PR-like protein isolated from wheat (Cruz and Ownby, 1993), the *parA* and *parB* genes of tobacco (Ezaki et al., 1995), and a gene encoding a putative peroxidase from tobacco suspension cultures (Ezaki et al., 1996). Besides being Al inducible, many of these genes are also up-regulated in response to other environmental stresses, including heavy metal toxicity, wounding, and salt stress. Since these genes are not exclusively regulated by Al stress, Snowden et al. (1995) concluded that aside from the primary effects of Al on the growing root, Al toxicity also results in the initiation of general stresses. The possibility that *als3* represents a delocalized stress response is appealing, but it is curious that the phenotype is not inducible by other metal stresses.

Recent work on Al inhibition of shoot growth of bean (*Phaseolus vulgaris*) cultivars revealed that the inhibition of leaf expansion in an Al-sensitive cultivar was also not necessarily associated with the hyperaccumulation of Al in the shoots, a situation similar to that in *als3* (Massot et al., 1992). Exposure of bean roots to inhibitory levels of Al caused an almost 10-fold increase in the accumulation of zeatin riboside in shoots, with only slight increases in the level of Al and no obvious changes in nutrient concentrations. This suggests that increased cytokinin levels may be at least partially responsible for the observed decreases in leaf growth.

Ethylene is often associated with abiotic stresses, but does not appear to play a role in the *als3* shoot phenotype. *Als3* seedlings do not show signs of a "triple response" when grown in the dark in the presence of Al (P.B. Larsen, personal observation). The triple response, particularly exaggerated apical hook development and inhibition of hypocotyl elongation, is a sensitive indicator of ethylene production in Arabidopsis seedlings (Guzmán and Ecker, 1990). Other phytohormones such as ABA may be involved in the shoot inhibition response. Phytohormones are suspect because there are many examples of their being involved in transmitting localized stress responses from roots to remote sites in the shoot (Jackson, 1997).

Ultimately, the isolation and characterization of the *als3* gene should resolve these issues and provide a better un-

Table III. *Dependence of als3 shoot inhibition on interaction with the root*

Treatment	Shoots with Delayed Leaf Enlargement
Wild-type seedlings	0/5
Wild-type excised shoots	0/5
als3 seedlings	5/5
als3 excised shoots	0/5

derstanding of the molecular and biochemical processes responsible for the Al-induced inhibition of root growth and leaf expansion.

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