Coordination between Apoplastic and Symplastic Detoxification Confers Plant Aluminum Resistance[1[C][W][OPEN]]

Xiao Fang Zhu, Gui Jie Lei, Zhi Wei Wang, Yuan Zhi Shi, Janet Braam, Gui Xin Li, and Shao Jian Zheng*

State Key Laboratory of Plant Physiology and Biochemistry, College of Life Sciences (X.F.Z., G.J.L., Z.W.W., S.J.Z.), and College of Agronomy and Biotechnology (G.X.L.), Zhejiang University, Hangzhou 310058, China; Department of Plant Physiology and Nutrition, Tea Research Institute, Chinese Academy of Agricultural Sciences, The Key Laboratory of Tea Chemical Engineering, Ministry of Agriculture, Hangzhou 310008, China (Y.Z.S.); and Department of Biochemistry and Cell Biology, Rice University, Houston, Texas 77005 (J.B.)

ORCID ID: 0000-0002-3336-8165 (S.J.Z.).

Whether aluminum toxicity is an apoplastic or symplastic phenomenon is still a matter of debate. Here, we found that three auxin overproducing mutants, *yucca*, the recessive mutant *superroot2*, and *superroot1* had increased aluminum sensitivity, while a transfer DNA insertion mutant, *xyloglucan endotransglucosylase/hydrolases15* (*xth15*), showed enhanced aluminum resistance, accompanied by low endogenous indole-3-acetic acid levels, implying that auxin may be involved in plant responses to aluminum stress. We used *yucca* and *xth15* mutants for further study. The two mutants accumulated similar total aluminum in roots and had significantly reduced cell wall aluminum and increased symplastic aluminum content relative to the wild-type ecotype Columbia, indicating that altered aluminum levels in the symplast or cell wall cannot fully explain the differential aluminum resistance of these two mutants. The expression of *Al sensitive1* (*ALS1*), a gene that functions in aluminum redistribution between the cytoplasm and vacuole and contributes to symplastic aluminum detoxification, was less abundant in *yucca* and more abundant in *xth15* than the wild type, consistent with possible ALS1 function conferring altered aluminum sensitivity in the two mutants. Consistent with the idea that *xth15* can tolerate more symplastic aluminum because of possible ALS1 targeting to the vacuole, morin staining of *yucca* root tip sections showed more aluminum accumulation in the cytosol than in the wild type, and *xth15* showed reduced morin staining of cytosolic aluminum, even though *yucca* and *xth15* had similar overall symplastic aluminum content. Exogenous application of an active auxin analog, naphthylacetic acid, to the wild type mimicked the aluminum sensitivity and distribution phenotypes of *yucca*, verifying that auxin may regulate aluminum distribution in cells. Together, these data demonstrate that auxin negatively regulates aluminum tolerance through altering *ALS1* expression and aluminum distribution within plant cells, and plants must coordinate exclusion and internal detoxification to reduce aluminum toxicity effectively.

Aluminum (Al) toxicity is a major growth-limiting factor for crop production on acid soils worldwide (Foy, 1988; Kochian, 1995), which occupy approximately 50% of the world's potential arable land (von Uexküll and Mutert, 1995). Ionic aluminum inhibits root elongation as well as water and nutrient uptake and results in significant loss of crop productivity (Kochian et al., 1995). Despite the increasing evidence of the functional and structural damage resulting from aluminum toxicity, the mechanism underlying aluminum-induced root growth inhibition remains unclear.

Aluminum-resistant plants have developed two mechanisms to cope with aluminum toxicity. One is based on the exclusion of aluminum from the root symplasm, whereas the other relies on the ability to tolerate symplastic aluminum (Taylor, 1991; Kochian et al., 2004). The well-documented exclusion mechanism is to prevent aluminum from entering root cells by secretion of organic acid anions by the root apex, resulting in the formation of stable nonphytotoxic chelates with aluminum (Kochian, 1995; Ryan et al., 2001; Ma and Furukawa, 2003). The internal detoxification of aluminum is primarily based on the storage of aluminum in the vacuole as aluminum-oxalate (Ma et al., 1997a; Shen et al., 2002) or aluminum-citrate (Ma et al., 1997b) complexes, thus changing the distribution of aluminum within cells (Ma, 2000; Ma and Hiradate, 2000). Recently, some transporters involved in the aluminum distribution within cells have been identified. In Arabidopsis (*Arabidopsis thaliana*), the tonoplast-localized ATP-binding cassette (ABC) transporter *Al sensitive1* (*ALS1*), which is involved in Al tolerance in Arabidopsis

[1] This work was supported by the Natural Science Foundation of China (grant no. 30830076), the Changjiang Scholarship and Innovative Research Team (IRT1185), the Fundamental Research Funds for Central Universities, and the U.S. National Science Foundation (grant no. 0313432 to J.B.).

* Corresponding author; e-mail sjzheng@zju.edu.cn.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Shao Jian Zheng (sjzheng@zju.edu.cn).

[C] Some figures in this article are displayed in color online but in black and white in the print edition.

[W] The online version of this article contains Web-only data.

[OPEN] Articles can be viewed online without a subscription.

www.plantphysiol.org/cgi/doi/10.1104/pp.113.219147

 © 2013 American Society of Plant Biologists. All Rights Reserved.

(Larsen et al., 2007), while *ALS3* is responsible for movement of aluminum away from sensitive tissues for sequestration in more tolerant tissues (Larsen et al., 2005, 2007). Huang et al. (2012) identified a rice (*Oryza sativa*) tonoplast-localized aluminum transporter, encoded by *OsALS1*, which is responsible for sequestration of aluminum into vacuoles and thus contributes to the internal detoxification of aluminum in rice. In addition, some plants have been found to harbor multiple strategies for aluminum detoxification. Buckwheat (*Fagopyrum esculentum*), for example, can secrete oxalate to detoxify external aluminum and accumulate large amounts of aluminum within vacuoles by forming aluminum-oxalate complexes in a molar ratio of 1:3 (Ma et al., 1997c). Rice, which is the most aluminum-resistant cereal crop, employs multiple strategies to achieve high aluminum resistance. The rice aluminum stress-responsive transcriptional factor, *ART1*, regulates expression of 31 downstream genes (Yamaji et al., 2009). In addition to gene-based aluminum tolerance strategies, some external factors can also affect aluminum resistance, such as external phytohormone application. For example, exogenous application of cytokinin can alleviate aluminum-induced inhibition of lateral root growth in aluminum-sensitive soybean (*Glycine max*; Pan et al., 2001). Several studies have demonstrated that aluminum may interact with auxin-signaling pathways, leading to alterations of auxin accumulation and distribution in roots (Kollmeier et al., 2000; Doncheva et al., 2005; Shen et al., 2008). A recent study indicated that Al^{3+} induced alteration of auxin distribution in roots, leading to arrest of root elongation, while naphthylphthalamic acid (an auxin polar transport inhibitor) applied exogenously substantially alleviated the aluminum-induced inhibition of root elongation (Sun et al., 2010). However, these previous studies only present circumstantial evidence on the disruption of the accumulation and polar transportation of auxin, which may be a primary cause of the aluminum-induced inhibition of root growth. The main mechanism of how auxin contributes to aluminum sensitivity remains unclear.

Cell walls are not only a critical site for aluminum storage in plants, but also serve as the first barrier to cellular aluminum uptake. For example, 85% to 90% of the total aluminum accumulated by barley (*Hordeum vulgare*) roots is tightly bound to the cell walls (Clarkson, 1967), and almost 90% of the cellular aluminum is associated with the cell walls of cultured tobacco (*Nicotiana tabacum*) cells (Chang et al., 1999). Accumulating evidence demonstrates that the cell wall plays important roles in the manifestation and perception of aluminum toxicity (Horst et al., 2010). The cell wall is very complex in structure and composition. The binding of aluminum changes cell wall structure, makes the wall more rigid, and reduces mechanical extensibility and cell expansion (Tabuchi and Matsumoto, 2001; Ma et al., 2004). An aluminum-sensitive rice cultivar accumulates higher aluminum in the cell wall than an aluminum-resistant cultivar (Yang et al., 2008). Higher pectin content is partially attributed to higher aluminum accumulation in the cell wall (Eticha et al., 2005a; Liu et al., 2008; Yang et al., 2011a). Furthermore, aluminum stress also results in an increase of not only pectin, but also hemicellulose content in wheat (*Triticum aestivum*; Tabuchi and Matsumoto, 2001), triticale (× *Triticosecale* Wittmack; Liu et al., 2008), and rice (Yang et al., 2008). Recently, Yang et al. (2011b) reported that hemicellulose, not pectin, is the major cell wall component that binds aluminum in Arabidopsis. Aluminum inhibits xyloglucan endotransglucosylase action, an enzyme that may cut and rejoin xyloglucan chains leading to cell wall loosening (Fry et al., 1992; Nishitani and Tominaga, 1992; Thompson and Fry, 2001), and down-regulates the expression of *xyloglucan endotransglucosylase/hydrolases* (*XTH14*), *XTH15*, and *XTH31*. Further study showed that Arabidopsis with *XTH31* knocked out has lower xyloglucan content and cell wall aluminum-binding capacity and higher aluminum resistance (Zhu et al., 2012). Aluminum-induced secretion of organic acid anions decreases aluminum retention in the cell wall (Zheng et al., 2004), and, as a consequence, aluminum content in the root apex is decreased, which renders plants more aluminum resistant. Together, these data support the view that higher aluminum retention in the cell wall results in greater aluminum sensitivity. However, an important question remains open; if the total aluminum content in the roots remains constant, but the aluminum retention in the cell wall is decreased, will plants be more aluminum sensitive or more aluminum resistant?

In this study, we used two Arabidopsis mutants that accumulate similar aluminum levels in the roots, but one mutant, *yucca*, is aluminum sensitive, whereas the other, *xth15*, is aluminum resistant. We explored the underlying mechanisms leading to this difference in aluminum sensitivity. Although both mutants accumulate similar aluminum levels in cell wall, *xth15* may sequester more aluminum into vacuoles than *yucca*. These data suggest the importance of coordination between external and internal detoxification mechanisms in Arabidopsis.

RESULTS

To investigate the effect of auxin on aluminum sensitivity, we used three high endogenous auxin mutants, the recessive mutants *superroot1* (*sur1-3*; Boerjan et al., 1995) and *sur2* (Delarue et al., 1998; Barlier et al., 2000) and the dominant activation-tagged *yucca1*, which overexpresses the flavin monooxygenase-like *YUCCA* proposed to be involved in Trp-dependent indole-3-acetic acid (IAA) biosynthesis (Zhao et al., 2001).

Inhibition of root elongation is the most typical symptom of aluminum toxicity in plants. When Arabidopsis seedlings were treated with 50 μM aluminum for 24 h, root elongation was inhibited by 63% in an auxin overproducing mutant, *yucca*, while 41% in the wild type (Fig. 1A). In longer-term (7-d) experiments, root growth was also more inhibited by exogenous aluminum in *yucca* than in the wild type (Fig. 1C). To

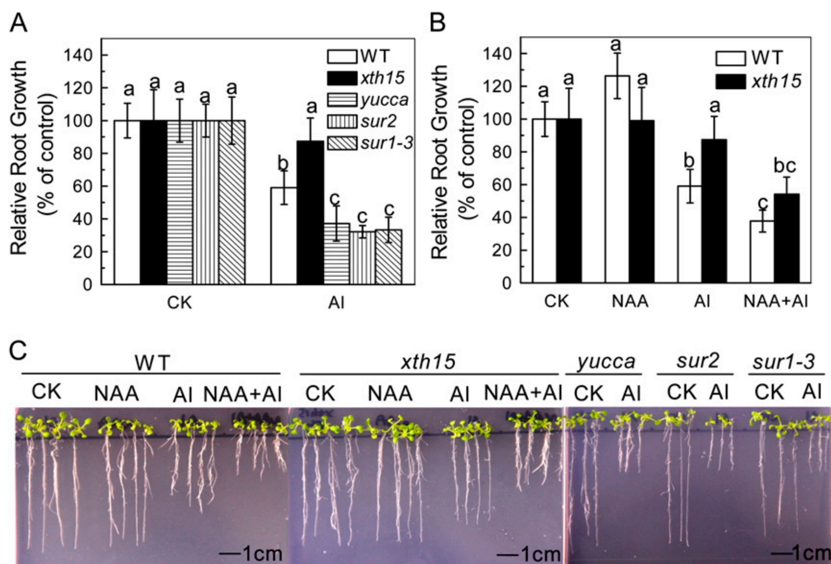

Figure 1. The effect of aluminum and NAA on root growth of Arabidopsis. A, One-centimeter-long seedlings were grown on 0.5 mM $CaCl_2$ medium containing 0 (CK) or 50 μM aluminum for 24 h. Data are means ± SD (n = 10). Columns with different letters are significantly different at $P < 0.05$. B, One-centimeter-long seedlings were grown on 0.5 mM $CaCl_2$ medium containing 0 or 50 μM aluminum in the presence or absence of NAA. Root elongation was measured before and after treatment for 24 h. Data are means ± SD (n = 10). Columns with different letters are significantly different at $P < 0.05$. C, The effect of aluminum and NAA on the root growth of Arabidopsis. One-centimeter-long seedlings were grown on nutrient plates containing 0 or 50 μM aluminum for 7 d. All the experiments were done at pH 4.5. Pictures were taken using a digital camera.

confirm that the aluminum-sensitive phenotypes observed in *yucca* are caused by the overproduction of auxin, we analyzed the aluminum sensitivity of the other two high endogenous auxin mutants, *sur2* and *sur1-3*, and found that they showed similar aluminum sensitivity as *yucca* (Fig. 1, A and C). As the increased endogenous auxin levels had similar effects on aluminum resistance, we used *yucca* as plants with "high levels of endogenous auxin" for the following experiments. Wild-type plants treated with naphthylacetic acid (NAA; an active auxin) also are more aluminum sensitive (Fig. 1, B and C), confirming that the aluminum sensitivity of *yucca* is likely due to auxin overaccumulation. We also measured the solution pH when 0.05 μM NAA was added to the nonbuffered aluminum solution and found that there was no decline of pH. Furthermore, when the solution was buffered at pH 4.5 with MES, the relative root elongation showed no significant difference with the nonbuffered solution (Fig. 1B; Supplemental Fig. S1). Moreover, if NAA increases the activity of aluminum, the inhibition of the root elongation should also be more profound in NAA plus aluminum treatment than aluminum treatment alone, but there was almost no difference of aluminum sensitivity in *yucca* between aluminum and NAA plus aluminum treatment (Supplemental Fig. S1). Therefore, the effect of NAA plus aluminum on root growth was caused by the synergetic action of aluminum and NAA. Furthermore, Figure 1A shows that *xth15*, mutants with a transfer DNA insertion in the *XTH15* locus, treated with 50 μM aluminum for 24 h showed only 13% root elongation inhibition compared with wild-type root inhibition of 41% (Fig. 1A). Similarly, *xth15* seedlings grown for 7 d on agar medium containing 50 μM Al^{3+} had longer roots than similarly treated wild-type seedlings (Fig. 1C), and the root growth of *xth15* was also inhibited by NAA plus aluminum (Fig. 1, B and C). Interestingly, the IAA levels in *xth15* roots were also lower than the wild type (Fig. 2) and correlated with enhanced aluminum resistance (Fig. 1). All these results demonstrate that higher levels of endogenous or exogenous auxin correlate with increased Arabidopsis sensitivity to aluminum.

Aluminum content is a critical index to indicate whether exclusion or internal detoxification mechanism underlies aluminum resistance. To distinguish between these mechanisms in the altered aluminum sensitivity of *yucca* and *xth15* mutants, we determined aluminum content in roots of seedlings exposed to 50 μM aluminum for 24 h. Surprisingly, the wild type, *yucca*, *xth15*, and the wild type and *xth15* treated with NAA all accumulated similar levels of aluminum (Fig. 3). These results suggest that elevated auxin or loss of *XTH15* function does not affect overall aluminum accumulation levels but may cause alterations in aluminum sensitivity by changing the distribution of aluminum within root cells.

To address cellular distribution, we first measured aluminum content in symplast and root cell walls according to Xia et al. (2010). The purity of the

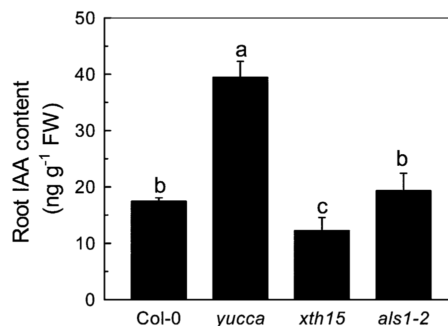

Figure 2. Accumulation of IAA in Col-0, *yucca*, *xth15*, and *als1-2* without aluminum treatment. Data are means ± SD (n = 4). Columns with different letters are significantly different at $P < 0.05$.

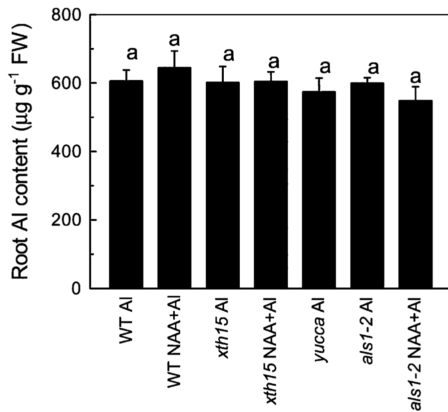

Figure 3. The aluminum content in the plant roots. Six-week-old plants were treated with 0.5 mM $CaCl_2$ solution containing 50 μM aluminum or 50 μM aluminum combined with 0.05 μM NAA for treatment with auxin applied exogenously. The pH was adjusted to 4.5. Data are means ± SD (n = 4). Columns with different letters are significantly different at $P < 0.05$.

apoplastic solution was reported by the absence of detectable malic dehydrogenase (about 35 kD; Fig. 4A). Aluminum accumulation in the symplast of the untreated wild type was lower than that in *yucca*, *xth15*, and the wild type treated with exogenous NAA (Fig. 4B), whereas the aluminum content in the cell wall was higher in the wild type than in the wild type treated with NAA or in *xth15* or *yucca* (Fig. 4C). Although the reduction in cell wall aluminum content of *xth15* relative to the wild type may correlate with the enhanced aluminum resistance of *xth15*, the reduction in cell wall aluminum of *yucca* and the NAA-treated wild type is unexpected, given the enhanced aluminum sensitivity of *yucca* and the NAA-treated wild type and *xth15*. These data prompted us to investigate whether differential subcellular localization of symplastic aluminum may impact the aluminum sensitivity phenotypes of *yucca*, *xth15*, and the wild type treated with exogenous NAA.

ALS1 is reported to be a root tip tonoplast transporter and responsible for aluminum redistribution between the cytoplasm and vacuole (Larsen et al., 2007). To determine whether differential expression abundance of *ALS1*, predicted to correlate with function levels, could underlie the differential sensitivity of *yucca* and *xth15*, we monitored *ALS1* expression in roots with and without aluminum stress. Although *ALS1* expression was not aluminum inducible (Fig. 5), in accordance with Larsen et al. (2007), root *ALS1* expression was about 50% lower in *yucca* and the wild type treated with exogenous NAA than in the wild type (Fig. 5) but was about 50% higher than the wild type in *xth15* under control conditions (Fig. 5). Therefore, relative *ALS1* expression correlated with resistance in these two mutants, suggesting that high auxin levels may impair *ALS1*-dependent aluminum detoxification, whereas loss of *XTH15*, which also resulted in a low auxin level, may enhance this subcellular detoxifying mechanism. Furthermore, we found that *als1-2* sensitivity to aluminum was not exacerbated by NAA treatment (Fig. 6, A and B), although NAA treatment of *als1-2* led to lower cell wall aluminum content (Fig. 6, C and D), consistent with the idea that auxin enhances aluminum toxicity by down-regulating the expression of *ALS1*.

To gain further evidence for possible vacuole compartmentalization of aluminum, we localized the aluminum that enters cells with morin staining. Morin can detect aluminum in the cytosol but not cell wall-bound aluminum or vacuole-compartmentalized aluminum (Eticha et al., 2005b; Huang et al., 2012). The lack of morin staining in vacuole may be attributed to two reasons according to Huang et al. (2012): (1) morin is not permeable to the tonoplast, and (2) vacuolar aluminum is chelated by organic reagents, such as malic and citric acids, and morin cannot detect complexed aluminum forms, similar to cell wall-bound aluminum (Eticha et al., 2005b). Therefore, strong aluminum-dependent green fluorescence represents aluminum present in the cytosol and nucleus. The green fluorescence of morin was only faintly detected when seedlings were treated with 0.5 mM $CaCl_2$ in the absence of

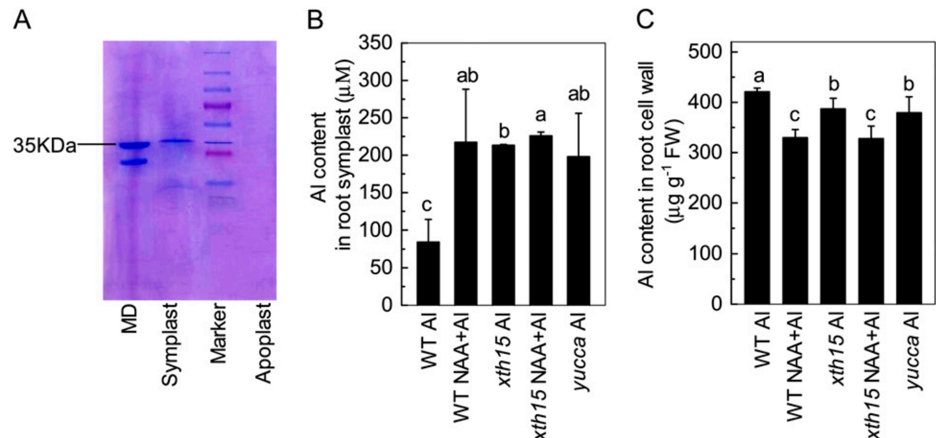

Figure 4. Purity of apoplastic solution (A), aluminum content in symplast (B), and cell wall (C). Six-week-old plants were grown on 0.5 mM $CaCl_2$ media containing 50 μM aluminum or 50 μM aluminum combined with 0.05 μM NAA for 24 h. The pH was adjusted to 4.5. Data are means ± SD (n = 4). Columns with different letters are significantly different at $P < 0.05$. MD, Malic dehydrogenase; Marker, protein standards. [See online article for color version of this figure.]

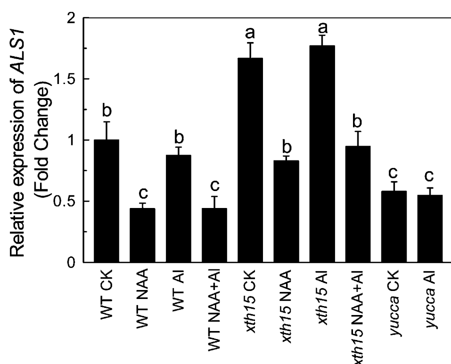

Figure 5. Effect of aluminum on *ALS1* expression. Six-week-old plants were treated with 0.5 mM $CaCl_2$ solution containing 0 or 50 μM aluminum in the presence or absence of 0.05 μM NAA for 24 h. The pH was adjusted to 4.5. Total RNAs were extracted from roots and subjected to reverse transcription followed by real-time PCR. Expression levels without aluminum treatment of the wild type were normalized to the expression level of *tubulin* under control conditions (minus aluminum) and were assigned an expression level of 1. Data are means of three independent biological replicates. Columns with different letters are significantly different at $P < 0.05$.

aluminum, whereas after exposure to 0.5 mM $CaCl_2$ combined with 50 μM aluminum, *yucca* and the NAA-treated wild type displayed stronger aluminum-dependent green fluorescence than the wild type in root cells (Fig. 7), and *xth15* displayed relatively weaker morin staining (Fig. 7). These results are consistent with the conclusion that elevated auxin results in more aluminum accumulation in the cytosol, whereas loss of *XTH15* function, with a lower level of endogenous auxin, accumulates less aluminum in the cytosol and therefore may target more aluminum to the vacuole.

DISCUSSION

To survive in an aluminum-toxic environment, aluminum-resistant plant species adopt strategies to either restrict aluminum uptake (exclusion of aluminum from the root symplasm) or cope with internalized aluminum (tolerate symplastic aluminum; Taylor, 1991; Kochian et al., 2004). The typical exclusion mechanism is generally associated with lower aluminum content in the roots or fixation of aluminum in the apoplast. However, in this study, although there is no significant difference in the total aluminum content among the roots of *yucca*, *xth15*, the NAA-treated wild type, and the non-NAA-treated wild type, there are large differences in aluminum resistance. To elucidate the possible mechanisms leading to the differential aluminum sensitivity, we found that although less aluminum retention in the cell wall may contribute to enhanced aluminum resistance, when similar amounts of aluminum are present in the roots, more exclusion of aluminum from the more susceptible sites such as cytoplasm and nucleus may also be fundamental for plant resistance to aluminum. This work provides solid evidence for the importance of cooperation between aluminum exclusion and internal detoxification in plants.

Increasing evidence has shown that binding of aluminum in cell wall appears to be closely related to aluminum sensitivity, as fixation of aluminum in the cell wall will affect the proper functioning of cell wall (Horst et al., 2010). For example, Horst (1995) reported that aluminum bound to cell wall components increases wall rigidity, affects cell wall loosening, and thus ultimately inhibits root elongation. Ma et al. (2004) demonstrated that aluminum decreases cell wall viscosity and elasticity, thus reducing cell wall extensibility, and, as a consequence, cell elongation is inhibited. Therefore, higher aluminum sensitivity is correlated with more aluminum accumulation in the cell wall, as demonstrated in maize (*Zea mays*) suspension cells (Schmohl and Horst, 2000) and intact root apices (Eticha et al., 2005a), rice (Yang et al., 2008), triticale (Liu et al., 2008), and rice bean (*Vigna umbellata*; Zhou et al., 2012). In Arabidopsis, there is also a large difference in aluminum resistance among different ecotypes (Hoekenga et al., 2006). Although there is no report on the difference of

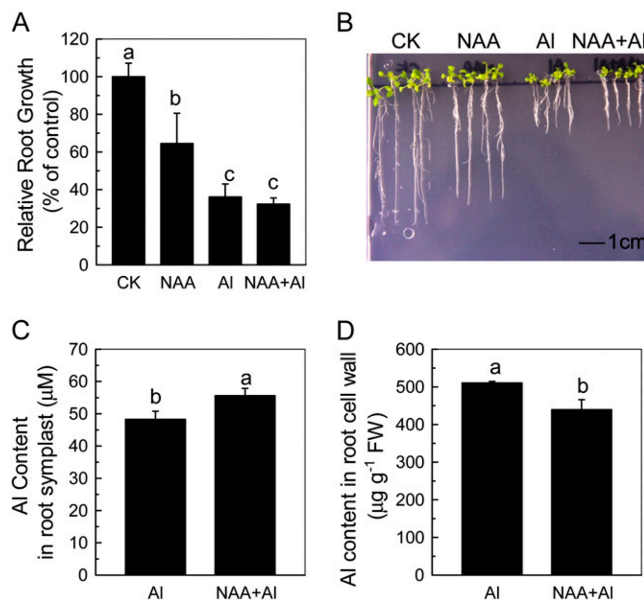

Figure 6. The effect of aluminum and NAA on the root growth and aluminum content in root symplast and cell wall of *als1-2*. A, One-centimeter-long seedlings were grown on 0.5 mM $CaCl_2$ medium containing 0 or 50 μM aluminum in the presence or absence of NAA for 24 h. The pH was adjusted to 4.5. Data are means ± SD (n = 10). Columns with different letters are significantly different at $P < 0.05$. B, The effect of aluminum and NAA on the root growth of Arabidopsis. One-centimeter-long seedlings were grown on nutrient plates containing 0 or 50 μM aluminum for 7 d. The pH was adjusted to 4.5. Pictures were taken using a digital camera. Aluminum content in symplast (C) and cell wall (D). Six-week-old plants were grown on 0.5 mM $CaCl_2$ solution containing 50 μM aluminum or 50 μM aluminum combined with 0.05 μM NAA for 24 h. The pH was adjusted to 4.5. Data are means ± SD (n = 4). Columns with different letters are significantly different at $P < 0.05$.

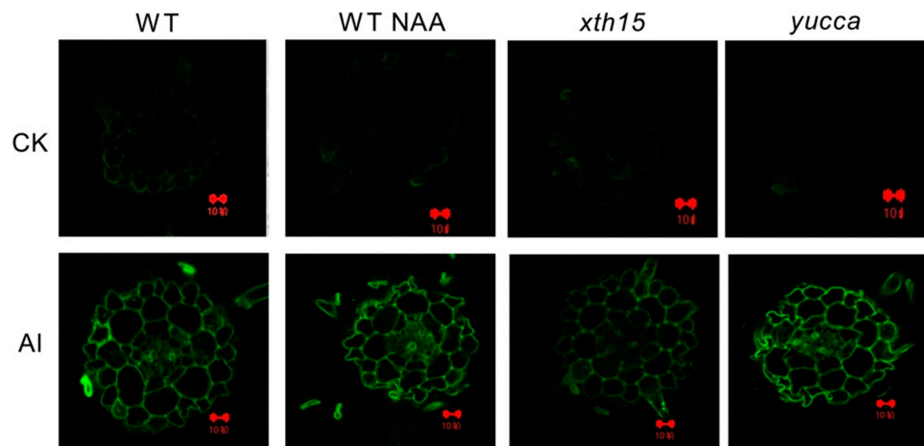

Figure 7. Subcellular distribution of aluminum stained with morin (green). About 1-cm-long seedlings were exposed to 0.5 mM $CaCl_2$ solution with or without 50 μM aluminum in the presence or absence of NAA for 12 h. The pH was adjusted to 4.5. Roots were transversely sectioned at 5 and 10 mm from the apex for morin staining and fluorescence observation. Bar = 10 μm.

aluminum content in roots among the different ecotypes, several studies demonstrated that aluminum-resistant mutants (Larsen et al., 1998) or transgenic lines (Ezaki et al., 2007) usually accumulate significantly less aluminum in the roots as compared with the wild type. Recently, we identified another Arabidopsis mutant *xth31* that is highly aluminum resistant. *xth31* accumulates significantly less aluminum in cell walls due to less xyloglucan content, as xyloglucan is responsible for aluminum binding in the hemicellulose of cell wall (Zhu et al., 2012). However, in some Arabidopsis mutants, aluminum sensitivity is not related to aluminum content. For example, *als7* and *als4* roots both accumulate less aluminum than the wild type after exposure to aluminum-containing solutions, yet root growth of the mutants is significantly inhibited (Larsen et al., 1996). Nezames et al. (2012) found that the increase in root growth seen for *aluminum tolerant 2* (*alt2-1*) *als3-1* compared with *als3-1* and ecotype Columbia (Col-0) under aluminum stress is not related to the root aluminum accumulation because *alt2-1 als3-1* roots accumulate wild-type levels of aluminum. Here, we screened a number cell wall-associated mutants for aluminum sensitivity and found a mutant, *xth15*, that displayed enhanced aluminum resistance. In this mutant, transfer DNA is inserted into the first exon of *XTH15* (110 bp downstream of the translation initiator ATG codon; Supplemental Fig. S2A). *XTH15* transcripts were not detected in the homozygous line (Supplemental Fig. S2B), indicating that the *xth15* allele is likely a null mutation. *XTH15* is likely to function in seedling roots because strong root staining is found in transgenics harboring a *GUS* reporter gene driven by the *XTH15* 5′ potential regulatory region (Becnel et al., 2006). We examined the two different mutants, the auxin-overproducing mutant, *yucca*, and the cell wall mutant, *xth15*, which showed large differences in aluminum sensitivity despite having similar root aluminum content. Furthermore, both mutants had moderate reductions of aluminum retention in the cell wall (Fig. 4C), suggesting that exclusion of aluminum from the cell wall is not necessarily sufficient to confer elevated aluminum resistance. Therefore, other mechanisms must exist that contribute to differential aluminum sensitivity.

A fundamental mechanism of internal detoxification is sequestration of aluminum into the vacuoles. Ma et al. (1997a, 1997c) identified forms of aluminum-organic acids compounds in the cell sap of hydrangea (*Hydrangea macrophylla*) and buckwheat leaves and proposed that these compounds might be sequestered in vacuoles. Later, Shen et al. (2002) demonstrated that most aluminum and oxalate in the protoplasts of buckwheat leaves is present in the vacuoles. These reports provide physiological evidence to demonstrate the compartmentalization of aluminum into the vacuole in the forms of aluminum-organic acids and the contribution of compartmentalization to internal aluminum detoxification. With the studies of various mutants altered in aluminum sensitivity, molecular mechanisms underlying resistance can be uncovered. Larsen et al. (2005) identified *ALS3*, which encodes a transporter that is localized to the phloem, leaf hydathodes, and root epidermis and is predicted to transport aluminum away from sensitive tissues for sequestration in more tolerant tissues. *als3* displays severe aluminum inhibition of root growth. In addition, *ALS1*, which encodes a transporter localized to the root tip and the vasculature, has been implicated in aluminum sequestration to more tolerant tissues (Larsen et al., 2007). Recently, Huang et al. (2012) identified that *OsALS1*, which is expressed ubiquitously in rice, with the encoded protein localizing to the tonoplast, is responsible for sequestration of aluminum into the vacuoles and is required for internal detoxification of aluminum in rice. Although both *ALS1* and *ALS3* have critical roles in aluminum resistance, *ALS3* is a plasma membrane transporter that moves aluminum away from the root tip and is not involved in redistribution between the cytoplasm and vacuole. We found, however, that *ALS1* expression was lower in *yucca* and the NAA-treated wild type but higher in *xth15* (Fig. 5), thus correlating with aluminum resistance. Moreover, the expression of *ALS1* was also down-regulated when *xth15* was treated with NAA (Fig. 5). However, there was lower IAA content in *xth15* roots, suggesting that auxin accumulation may affect *ALS1* expression and

altered *ALS1* expression may affect the efficacy of internal detoxifying mechanisms of aluminum in plants, which is in accordance with the no effect of NAA in the *als1-2* mutant (Fig. 6). However, it is interesting that the NAA applied exogenously had no effect on the wild type and *xth15* growth, while it inhibited the *als1-2* growth (Fig. 1 and 6); this may be attributed to differential sensitivity to NAA, and *als1-2* might have a lower suitable NAA level to promote the root growth. Furthermore, the morin staining reported more aluminum present in the cytosol in *yucca* and less cytosolic aluminum in *xth15* than the wild type (Fig. 7). Therefore, when similar amounts of aluminum are present in the symplasm, there may be enhanced aluminum redistribution into the vacuoles facilitated by enhanced expression of *ALS1*, which may lead to more aluminum resistance. These results lead to the next question of how might auxin regulate the expression of *ALS1*. As there are no auxin response elements TGTCTC in the 2 kb-promoter regions of *ALS1* genes (Hagen and Guilfoyle, 2002), the effect may be indirect, needing further investigation.

In this study, we also demonstrated that auxin is a negative factor in plant aluminum resistance, as both endogenous auxin overproduction, as in the *yucca* mutant, and exogenous application of NAA resulted in higher aluminum sensitivity in Arabidopsis, whereas *xth15* with low levels of endogenous auxin exhibited higher aluminum resistance (Fig. 1). We have used a series of auxin concentrations and found that with each incremental increase in the auxin concentration, *ALS1* expression was progressively repressed, accompanied by increasing inhibition of root growth (Supplemental Fig. S3). Kollmeier et al. (2000) found that aluminum alters auxin accumulation and distribution in roots possibly due to effects on the auxin polar transport system, while application of exogenous IAA to the elongation zone significantly alleviated the aluminum-induced inhibition of root growth in maize. Recently, Sun et al. (2010) demonstrated that aluminum affects auxin distribution through aluminum-induced changes in ethylene production; however, application of an IAA polar transport inhibitor can partially alleviate the inhibition of root growth in Arabidopsis under aluminum stress. The role of auxin in aluminum resistance is therefore complex. Effects may be influenced by species-specific responses or experimental treatment conditions. However, previous studies reported a relationship between aluminum-induced inhibition of root elongation and the disruption of auxin accumulation or distribution; the underlying physiological and molecular mechanisms remained undefined. Here, we demonstrate that auxin may exacerbate aluminum sensitivity by modifying the expression of *ALS1* and therefore aluminum redistribution, as seen in *xth15*, *yucca*, and the wild type supplied with exogenous NAA (Fig. 5; Supplemental Fig. S3). Therefore, auxin may have a role in altering aluminum distribution within cells.

In conclusion, our study focusing on the aluminum resistance of the wild type, *yucca*, and *xth15* demonstrates that auxin negatively regulates aluminum tolerance through altering *ALS1* expression and aluminum distribution within plant cells, providing evidence for the importance for plant coordination of apoplastic and symplastic detoxification of aluminum to withstand aluminum toxicity.

MATERIALS AND METHODS

Plant Material and Growth Conditions

The Col-0 of Arabidopsis (*Arabidopsis thaliana*) served as the wild type and the background for all mutants, including *xth15*, *yucca*, *sur2*, *sur1-3*, and the aluminum-sensitive mutant *als1-2* used in this study. For short-term (24-h) treatments, 0.5 mM $CaCl_2$ solution (pH 4.5) was used as control media, while for longer-duration (7-d) treatments, the nutrient solution (pH 4.5) was used as control media and 50 μM aluminum and 0.05 μM NAA were directly added for aluminum, NAA, or NAA plus aluminum treatments. Seeds were surface sterilized and germinated on an agar-solidified nutrient medium in petri dishes. The nutrient medium consisted of the macronutrients 6.0 mM KNO_3, 4.0 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, and 0.1 mM $NH_4H_2PO_4$ and the micronutrients 50 μM Fe(III)-EDTA, 12.5 μM H_3BO_3, 1 μM $MnSO_4$, 0.5 μM $CuSO_4$, 1 μM $ZnSO_4$, 0.1 μM H_2MoO_4, and 0.1 μM $NiSO_4$ according to Murashige and Skoog salts (Murashige and Skoog, 1962). The final pH was adjusted to 4.5. The seeds were vernalized at 4°C for 2 d. Petri dishes were placed into a growth chamber, positioned vertically, and kept under controlled environmental conditions at 24°C, 140 μmol photons m^{-2} s^{-1}, and a 16-h/8-h day/night rhythm.

For hydroponic culture, seedlings were first aseptically germinated on the above solid Murashige and Skoog medium. After 2 weeks, the young plantlets were placed on vermiculite for additional 3 weeks in an environmentally controlled growth chamber. Seedlings of similar rosette diameters were then transferred to the nutrient solution containing Murashige and Skoog salts for another week. Then the plants were subjected to the following treatments: control media (0.5 mM $CaCl_2$, pH 4.5), aluminum (50 μM aluminum in the 0.5 mM $CaCl_2$, pH 4.5), NAA (adding 0.05 μM NAA to the above-mentioned control media solution), and NAA plus aluminum (adding 0.05 μM NAA to the above-mentioned aluminum solution). After 24 h, the roots were excised for RNA extraction or for aluminum content analysis. When for aluminum content analysis, the seedlings were washed three times with deionized water, and the fresh weight was recorded.

Effect of Aluminum on Root Growth

Seedlings with root lengths of 1 cm were selected and transferred to petri dishes containing agar-solidified $CaCl_2$ (0.5 mM) medium with different aluminum concentrations (0 and 50 μM total concentration of aluminum in the form of $AlCl_3.6H_2O$). Root length measurements were performed using a digital camera connected to a computer. Data were quantified and analyzed by Photoshop 7.0 (Adobe Systems). For long-duration experiments, seedlings with a root length of 1 cm were selected and then transferred to petri dishes containing agar-solidified nutrient solution medium with different aluminum concentrations (0 and 50 μM total concentration of aluminum).

Gene Expression Analysis

Total RNA was isolated using TRIzol (Invitrogen). Complementary DNA was prepared from 1 μg of total RNA using the PrimeScript RT reagent kit (Takara). For real-time reverse transcription-PCR analysis, 1 μL of 10-fold-diluted complementary DNA was used for the quantitative analysis of gene expression performed with SYBR Premix ExTaq (Takara), with the following pairs of gene-specific primers: *ALS1*, forward, 5′-GACCGTTGG-AGCACTCACTTC-3′ and reverse, 5′-CAGGATTACCGACTGGACACT-3′ and for *tubulin*, forward, 5′-AAGTTCTGGGAAGTGGTT-3′ and reverse, 5′-CTCCCAATGAGTGACAAA-3′. Each complementary DNA sample was run in triplicate. Expression data were normalized with the expression level of *tubulin* gene. For the semiquantitative reverse transcription-PCR analysis, the primers used were as follows: for *18S*, forward, 5′-ATGATAACTCGACG-GATCGC-3′ and reverse, 5′-CTTGGATGTGGTAGCCGTTT-3′ and for *XTH15*, forward, 5′-CCGCTCGAGAAGAGAAGCAACTTCTTCGACGAGT-3′ and reverse, 5′-GCTCTAGAGACTCTGGACTTCTTGCATTCTGG-3′.

Aluminum Content Measurement

After treatment, the roots were excised after washing three times with 0.5 mM $CaCl_2$ and then put in Ultrafree-MC Centrifugal Filter Units (Millipore) and centrifuged at 3,000*g* for 10 min at 4°C to remove apoplastic solution. The roots were then frozen at –80°C overnight. The root cell sap solution was obtained by thawing the samples at room temperature and then centrifuging at 20,600*g* for 10 min. The residual cell walls were washed with 70% (v/v) ethanol three times before being immersed in 0.5 mL of 2 N HCl for 36 h with occasional vortexing, according to Xia et al. (2010). For root aluminum content analysis, materials were digested with HNO_3:$HClO_4$ (4:1, v/v). The aluminum in the root, symplastic solution, and cell wall extracts was determined by inductively coupled plasma-atomic emission spectrometry (IRIS/AP optical emission spectrometer).

SDS-PAGE

The protein from the apoplastic and root cell sap fractions and malic dehydrogenase (*Thermus flavus*; Sigma, M7032) were heated at 100°C for 10 min in 2×SDS-loading buffer to denature the proteins. Twenty microliters of the protein samples were loaded in each well.

SDS-PAGE was conducted using a 12% (w/v) resolving gel and 4% (w/v) stacking gel. The resolving gel, to a total volume of 10 mL, consisted of 3.35 mL water (distilled), 4 mL 30% (w/w) acrylamide/bis-acrylamide stock, 2.5 mL 1.5 M Tris-HCl (pH 8.8), 0.1 mL 10% (w/v) SDS, 0.05 mL 10% (w/v) ammonium persulfate, and 0.005 mL N,N,N′,N′-tetramethylethylenediamine, and was then carefully poured into glass plate and overlay gel with distilled, deionized H_2O to ensure a flat surface and to exclude air. After the gel has set more than half an hour, we got rid of the distilled, deionized H_2O, mixed the 4% stacking gel (3.05 mL water [distilled], 0.65 mL 30% acrylamide/bis-acrylamide stock, 1.25 mL 0.5 M Tris-HCl [pH 6.8], 0.05 mL 10% (w/v) SDS, 0.025 mL 10% ammonium persulfate, and 0.005 mL N,N,N′,N′-tetramethylethylenediamine), poured it onto the top of the set resolving gel, and inserted the comb. The electrophoresis buffer consisted of 3.03 g L^{-1} Tris base, 18.77 g L^{-1} Gly, and 1 g L^{-1} SDS. The gel runs at 80 V for about 3 h. The gel was stained with 0.1% (w/v) Coomassie Brilliant Blue (R250) in methanol:water:acetic acid (45:45:10, v/v/v) for 2 to 3 h at room temperature with agitation and then destained in ethanol:water:acetic acid (45:45:10, v/v/v). Finally, pictures were taken using a digital camera.

Morin Staining

About 1-cm-long seedlings of the wild type (Col-0) and the *yucca* and *xth15* mutants were exposed to 0.5 mM $CaCl_2$ solution (pH 4.5) containing 50 μM aluminum for 12 h. Roots were stained in 0.01% morin for 30 min, then excised and embedded in 5% (w/v) agar. Root tips were transversely sectioned from the apex, and the green fluorescence signal was observed using a laser-scanning confocal microscope (LSM510, Zeiss).

IAA Measurement

For analysis of the IAA concentration in roots, the whole root (about 20 mg) was collected for each sample. Four replicates of the samples were purified after the addition of 250 picograms ^{13}C6-IAA internal standard and analyzed by gas chromatography-selected reaction monitoring mass spectrometry as described (Ljung et al., 2005).

Statistical Analysis

Each experiment was repeated at least three times. Data were analyzed by one-way ANOVA procedure, and the means were compared by Duncan's multiple range test. Different letters on the histograms indicate that the means were statistically different at $P < 0.05$ level.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure 1. The effect of aluminum on root growth of Arabidopsis.

Supplemental Figure 2. Schematic structure of the *xth15* mutant carrying a single copy of the transfer DNA insert in the first exon of the *XTH15* gene.

Supplemental Figure 3. The effect of aluminum and NAA on root growth of Arabidopsis.

ACKNOWLEDGMENTS

We thank Catherine Bellini (University of Nottingham) for the *sur2* and *sur1-3* seeds, Yunde Zhao (University of California, San Diego) for the *yucca* seeds, and two anonymous reviewers for their valuable comments to improve the quality of our work.

Received April 3, 2013; accepted June 14, 2013; published June 19, 2013.

LITERATURE CITED

Barlier I, Kowalczyk M, Marchant A, Ljung K, Bhalerao R, Bennett M, Sandberg G, Bellini C (2000) The SUR2 gene of *Arabidopsis thaliana* encodes the cytochrome P450 CYP83B1, a modulator of auxin homeostasis. Proc Natl Acad Sci USA **97:** 14819–14824

Becnel J, Natarajan M, Kipp A, Braam J (2006) Developmental expression patterns of Arabidopsis *XTH* genes reported by transgenes and Genevestigator. Plant Mol Biol **61:** 451–467

Boerjan W, Cervera M-T, Delarue M, Beeckman T, Dewitte W, Bellini C, Caboche M, Van Onckelen H, Van Montagu M, Inzé D (1995) *Superroot*, a recessive mutation in *Arabidopsis*, confers auxin overproduction. Plant Cell **7:** 1405–1419

Chang YC, Yamamoto Y, Matsumoto H (1999) Accumulation of aluminium in the cell wall pectin in cultured tobacco (*Nicotiana tabacum* L.) cells treated with a combination of aluminium and iron. Plant Cell Environ **22:** 1009–1017

Clarkson DT (1967) Interactions between aluminum and phosphorus on root surfaces and cell wall material. Plant Soil **27:** 347–356

Delarue M, Prinsen E, Onckelen HV, Caboche M, Bellini C (1998) Sur2 mutations of *Arabidopsis thaliana* define a new locus involved in the control of auxin homeostasis. Plant J **14:** 603–611

Doncheva S, Amenós M, Poschenrieder C, Barceló J (2005) Root cell patterning: a primary target for aluminium toxicity in maize. J Exp Bot **56:** 1213–1220

Eticha D, Stass A, Horst WJ (2005a) Cell-wall pectin and its degree of methylation in the maize root-apex: significance for genotypic differences in aluminium resistance. Plant Cell Environ **28:** 1410–1420

Eticha D, Stass A, Horst WJ (2005b) Localization of aluminium in the maize root apex: can morin detect cell wall-bound aluminium? J Exp Bot **56:** 1351–1357

Ezaki B, Kiyohara H, Matsumoto H, Nakashima S (2007) Overexpression of an auxilin-like gene (F9E10.5) can suppress Al uptake in roots of Arabidopsis. J Exp Bot **58:** 497–506

Foy CD (1988) Plant adaptation to acid, aluminum-toxic soils. Commun Soil Sci Plant **19:** 959–987

Fry SC, Smith RC, Renwick KF, Martin DJ, Hodge SK, Matthews KJ (1992) Xyloglucan endotransglycosylase, a new wall-loosening enzyme activity from plants. Biochem J **282:** 821–828

Hagen G, Guilfoyle T (2002) Auxin-responsive gene expression: genes, promoters and regulatory factors. Plant Mol Biol **49:** 373–385

Hoekenga OA, Maron LG, Piñeros MA, Cançado GMA, Shaff J, Kobayashi Y, Ryan PR, Dong B, Delhaize E, Sasaki T, et al (2006) *AtALMT1*, which encodes a malate transporter, is identified as one of several genes critical for aluminum tolerance in Arabidopsis. Proc Natl Acad Sci USA **103:** 9738–9743

Horst WJ (1995) The role of the apoplast in aluminium toxicity and resistance of higher plants: a review. Z Pflanzenernähr Bodenkd **158:** 419–428

Horst WJ, Wang Y, Eticha D (2010) The role of the root apoplast in aluminium-induced inhibition of root elongation and in aluminium resistance of plants: a review. Ann Bot (Lond) **106:** 185–197

Huang CF, Yamaji N, Chen Z, Ma JF (2012) A tonoplast-localized half-size ABC transporter is required for internal detoxification of aluminum in rice. Plant J **69:** 857–867

Kochian LV (1995) Cellular mechanisms of aluminum toxicity and resistance in plants. Annu Rev Plant Physiol Plant Mol Biol **46:** 237–260

Kochian LV, Hoekenga OA, Piñeros MA (2004) How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. Annu Rev Plant Biol **55:** 459–493

Kollmeier M, Felle HH, Horst WJ (2000) 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 **122:** 945–956

Larsen PB, Cancel J, Rounds M, Ochoa V (2007) *Arabidopsis* ALS1 encodes a root tip and stele localized half type ABC transporter required for root growth in an aluminum toxic environment. Planta **225:** 1447–1458

Larsen PB, Degenhardt J, Tai CY, Stenzler LM, Howell SH, Kochian LV (1998) Aluminum-resistant Arabidopsis mutants that exhibit altered patterns of aluminum accumulation and organic acid release from roots. Plant Physiol **117:** 9–18

Larsen PB, Geisler MJ, Jones CA, Williams KM, Cancel JD (2005) ALS3 encodes a phloem-localized ABC transporter-like protein that is required for aluminum tolerance in *Arabidopsis*. Plant J **41:** 353–363

Larsen PB, Tai CY, Kochian LV, Howell SH (1996) Arabidopsis mutants with increased sensitivity to aluminum. Plant Physiol **110:** 743–751

Liu Q, Yang JL, He LS, Li YY, Zheng SJ (2008) Effect of aluminum on cell wall, plasma membrane, antioxidants and root elongation in triticale. Biol Plant **52:** 87–92

Ljung K, Hull AK, Celenza J, Yamada M, Estelle M, Normanly J, Sandberg G (2005) Sites and regulation of auxin biosynthesis in *Arabidopsis* roots. Plant Cell **17:** 1090–1104

Ma JF (2000) Role of organic acids in detoxification of aluminum in higher plants. Plant Cell Physiol **41:** 383–390

Ma JF, Furukawa J (2003) Recent progress in the research of external Al detoxification in higher plants: a minireview. J Inorg Biochem **97:** 46–51

Ma JF, Hiradate S (2000) Form of aluminium for uptake and translocation in buckwheat (*Fagopyrum esculentum Moench*). Planta **211:** 355–360

Ma JF, Hiradate S, Nomoto K, Iwashita T, Matsumoto H (1997a) Internal detoxification mechanism of Al in hydrangea. Identification of Al form in the leaves. Plant Physiol **113:** 1033–1039

Ma JF, Hiradate S, Nomoto K, Iwashita T, Matsumoto H (1997b) Internal detoxification mechanism of Al in hydrangea (identification of Al form in the leaves). Plant Physiol **113:** 1033–1039

Ma JF, Shen RF, Nagao S, Tanimoto E (2004) Aluminum targets elongating cells by reducing cell wall extensibility in wheat roots. Plant Cell Physiol **45:** 583–589

Ma JF, Zheng SJ, Hiradate S, Matsumoto H (1997c) Detoxifying aluminium with buckwheat. Nature **390:** 569–570

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant **15:** 473–496

Nezames CD, Sjogren CA, Barajas JF, Larsen PB (2012) The *Arabidopsis* cell cycle checkpoint regulators TANMEI/ALT2 and ATR mediate the active process of aluminum-dependent root growth inhibition. Plant Cell **24:** 608–621

Nishitani K, Tominaga R (1992) Endo-xyloglucan transferase, a novel class of glycosyltransferase that catalyzes transfer of a segment of xyloglucan molecule to another xyloglucan molecule. J Biol Chem **267:** 21058–21064

Pan JW, Zhu MY, Chen H (2001) Aluminum-induced cell death in root-tip cells of barley. Environ Exp Bot **46:** 71–79

Ryan PR, Delhaize E, Jones DL (2001) Function and mechanism of organic anion exudation from plant roots. Annu Rev Plant Physiol Plant Mol Biol **52:** 527–560

Schmohl N, Horst WJ (2000) Cell wall pectin content modulates aluminium sensitivity of *Zea mays* (L.) cells grown in suspension culture. Plant Cell Environ **23:** 735–742

Shen H, Hou NY, Schlicht M, Wan YL, Baluska F (2008) Aluminum toxicity targets PIN2 in Arabidopsis root apices: effects on PIN2 endocytosis vesicular recycling, and polar auxin transport. Chin Sci Bull **53:** 2480–2487

Shen RF, Ma JF, Kyo M, Iwashita T (2002) Compartmentation of aluminium in leaves of an Al-accumulator, *Fagopyrum esculentum* Moench. Planta **215:** 394–398

Sun P, Tian QY, Chen J, Zhang WH (2010) Aluminium-induced inhibition of root elongation in Arabidopsis is mediated by ethylene and auxin. J Exp Bot **61:** 347–356

Tabuchi A, Matsumoto H (2001) Changes in cell-wall properties of wheat (*Triticum aestivum*) roots during aluminum-induced growth inhibition. Physiol Plant **112:** 353–358

Taylor GJ (1991) Current views of the aluminum stress response: the physiological basis of tolerance. Curr Top Plant Biochem Physiol **10:** 57–93

Thompson JE, Fry SC (2001) Restructuring of wall-bound xyloglucan by transglycosylation in living plant cells. Plant J **26:** 23–34

von Uexküll HR, Mutert E (1995) Global extent, development and economic impact of acid soils. *In* RA Date, NJ Grundon, GE Raymet, ME Probert, eds, Plant-Soil Interactions at Low pH: Principles and Management. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 5–19

Xia JX, Yamaji N, Kasai T, Ma JF (2010) Plasma membrane-localized transporter for aluminum in rice. Proc Natl Acad Sci USA **107:** 18381–18385

Yamaji N, Huang CF, Nagao S, Yano M, Sato Y, Nagamura Y, Ma JF (2009) A zinc finger transcription factor ART1 regulates multiple genes implicated in aluminum tolerance in rice. Plant Cell **21:** 3339–3349

Yang JL, Li YY, Zhang YJ, Zhang SS, Wu YR, Wu P, Zheng SJ (2008) Cell wall polysaccharides are specifically involved in the exclusion of aluminum from the rice root apex. Plant Physiol **146:** 602–611

Yang JL, Zhu XF, Peng YX, Zheng C, Li GX, Liu Y, Shi YZ, Zheng SJ (2011b) Cell wall hemicellulose contributes significantly to aluminum adsorption and root growth in Arabidopsis. Plant Physiol **155:** 1885–1892

Yang JL, Zhu XF, Zheng C, Zhang YJ, Zheng SJ (2011a) Genotypic differences in Al resistance and the role of cell-wall pectin in Al exclusion from the root apex in *Fagopyrum tataricum*. Ann Bot (Lond) **107:** 371–378

Zhao Y, Christensen SK, Fankhauser C, Cashman JR, Cohen JD, Weigel D, Chory J (2001) A role for flavin monooxygenase-like enzymes in auxin biosynthesis. Science **291:** 306–309

Zheng SJ, Lin XY, Yang JL, Liu Q, Tang CX (2004) The kinetics of aluminum adsorption and desorptiion by root cell walls of an aluminum resistant wheat (*Triticum aestivum* L.) cultivar. Plant Soil **261:** 85–90

Zhou Y, Xu XY, Chen LQ, Yang JL, Zheng SJ (2012) Nitric oxide exacerbates Al-induced inhibition of root elongation in rice bean by affecting cell wall and plasma membrane properties. Phytochemistry **76:** 46–51

Zhu XF, Shi YZ, Lei GJ, Fry SC, Zhang BC, Zhou YH, Braam J, Jiang T, Xu XY, Mao CZ, et al (2012) *XTH31*, encoding an in-vitro XEH/XET-active enzyme, controls Al sensitivity by modulating in-vivo XET action, cell wall xyloglucan content and Al binding capacity in *Arabidopsis*. Plant Cell **24:** 4731–4747