J Plant Growth Regul (2008) 27:19–25
DOI 10.1007/s00344-007-9026-3

Abscisic Acid Response of Corn (*Zea mays* L.) Roots and Protoplasts to Lanthanum

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Received: 19 April 2007 / Accepted: 5 June 2007 / Published online: 10 October 2007

Abstract Lanthanum ions antagonize calcium and are used as a Ca^{2+} channel blocker but their direct effects are unknown. We investigated lanthanum effects on endogenous abscisic acid (ABA) levels in protoplasts and intact primary roots of *Zea mays* L. Application of 1 mM La^{3+} reduced primary root elongation, caused swelling of root tips, and essentially doubled the ABA content in intact roots but decreased ABA in root protoplasts in a concentration-dependent manner. Osmotic stress increased ABA level in protoplasts more than in intact roots. Temporal ABA changes in response to La^{3+} treatment indicate that La^{3+} affects root growth at least partially via ABA pathway.

Keywords Abscisic acid · Lanthanum · Roots · Protoplasts

Introduction

Lanthanides inhibit ion channels in plants and animals and have been used extensively as calcium antagonists. With an ionic radius similar to Ca^{2+} but with a higher charge density, La^{3+} competes for Ca^{2+}-binding sites on the plasma membrane (dos Remedios 1981; Martin and Richardson 1979; Thomson and others 1973). Rare earth elements like La^{3+} favor growth and crop productivity at low concentrations (He and Loh 2000) but are often inhibitory for root growth (Diatloff and others 1995; for review see Tyler 2005). Studies on La^{3+} have focused on its function as a calcium channel blocker. Lanthanum was used as an inhibitor to demonstrate the mechanism for calcium influx (Gelli and Blumwald 1997), Ca^{2+}-mediated signaling (Ding and Pickard 1993; Fasano and others 2002), and gene expression such as in response to drought and salinity (Knight and others 1997).

Contrary to the notion that diffusion of lanthanum is limited by the apoplastic barrier (Lehmann and others 2000; Thomson and others 1973), lanthanides enter the symplasm and affect plant growth (Liu and Hasenstein 2005; Quiquampoix and others 1990; Van Steveninck and others 1976). The uptake of La^{3+} in maize root protoplasts was concentration and time dependent and interfered with the cytoskeletal organization (Liu and Hasenstein 2005). Lanthanide ions are agonists of abscisic acid (ABA)-inducible gene expression and activated ABA-inducible promoters in rice protoplasts, and act upstream of phospholipase that is involved in the ABA signaling pathway (Hagenbeek and others 2000). In addition, lanthanum showed synergistic interaction with ABA and overexpressed VP1 (*Vivi*parous-*1*, a transcriptional activator; Gampala and others 2001) in rice protoplasts. These data suggest that lanthanum affects plant growth via the stabilization of the cytoskeleton (Liu and Hasenstein 2005) and/or by its synergistic and agonistic interaction with the stress hormone ABA (Gampala and others 2001; Hagenbeek and others 2000; Rock and Quatrano 1996).

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ABA serves as an important (stress) signal that regulates growth (Finkelstein and others 2002) and accumulates in tissues subjected to osmotic, salinity, desiccation, cold, and heavy metal stresses (Christmann and others 2006; Xie and others 2005; Xiong and others 2002). In maize ABA transiently promoted water conductivity of roots and permeability of cortex cells (Hose and others 2000) and triggered the expression of ABA-responsive genes that maintain root development and growth under stress (Finkelstein and others 2002). Because La^{3+} is taken up into protoplasts and inhibits root growth (Liu and Hasenstein 2005; Quiquampoix and others 1990), we used root protoplasts to study La^{3+} and ABA responses under different conditions. Isolated protoplasts retain their identity and differentiated state (Sheen 2001). However, the functions or effects of ABA in isolated protoplasts are unclear. Isolated protoplasts from rose petals and mesophyll cells of *Vicia faba* accumulated ABA under high osmolarity (Bianco-Trinchant and others 1993; Weiler and others 1982), but the ABA content of barley leaf protoplasts did not change when subjected to osmotic stress (Loveys and Robinson 1987). These studies suggest that the cell wall or cell–cell interactions may be required for the transduction of signals that trigger ABA synthesis (Bianco-Trinchant and Le Page-Degivry 1998). The present study shows the influence of La^{3+} on endogenous ABA levels in both intact roots and protoplasts. Because physiologic stressors and La^{3+} may exert at least part of their effects via the ABA signaling pathway, we also investigated if La^{3+} mediates the transcription of *ABA1*, a key gene encoding zeaxanthin epoxidase that catalyzes the epoxidation of zeaxanthin to antheraxanthin and violaxanthin (Duckham and others 1991; Barrero and others 2005). In addition to providing insight into the mechanism of La^{3+} on root physiology and (adaptation to) metal stress, our data illustrate the complexity of protoplasts as a physiologic tool.

Materials and Methods

Plant Material and Growth Condition

Seeds of maize (*Zea mays* L. cv Pioneer 3085) were soaked in deionized water overnight, planted between wet paper towels in plastic trays, and grown vertically in the dark at 24°C for about 2 days. Seedlings with 2-3-cm-long straight primary roots were used for the experiments.

Measurement of Root Growth

Maize seedlings were transferred to plastic Petri dishes and mounted vertically between filter papers wetted with 5 mM Mes/Tris buffer (pH 6.2). Images were taken (Nikon Coolpix 4500) after 3, 5, 8, 12, and 24 h of growth. The root length and diameter were measured with Image J (NIH, ver. 1.32j) software. Root diameter was measured at the meristematic region, 3 mm from the root tip, after 24 h of La^{3+} treatment. The growth rate was calculated based on the growth increments between measurements. Each treatment was measured twice with three repeats each.

Isolation of Protoplasts

Protoplasts were prepared from 2-3-cm-long primary roots, sliced longitudinally, and digested overnight in 20 ml osmoticum [0.6 M mannitol, 5 mM Mes/Tris (pH 6.0), 1 mM $CaCl_2$, 0.2 mM $MgCl_2$] containing 2% cellulase, 0.2% pectolyase Y-23 (Kikkoman Corp., Japan), and 0.05% BSA. Protoplasts were filtered through 85-μm polypropylene mesh and centrifuged (200*g*, 10 min). The sedimented protoplasts were resuspended in osmoticum without enzymes and layered on top with osmoticum containing 0.6 M sucrose instead of mannitol. After centrifugation (90*g*, 10 min), protoplasts at the interphase were collected and washed three times in osmoticum without $CaCl_2$. Protoplasts were pelleted (130*g*, 5 min), resuspended in osmoticum, and subdivided into aliquots.

Vital Staining of Protoplasts

Fluorescein diacetate (5 mg in 1 ml acetone) was mixed with 20 ml osmoticum [0.6 M mannitol, 5 mM Mes/Tris (pH 6.0), 1 mM $CaCl_2$, 0.2 mM $MgCl_2$]. Of this stock solution 0.25 ml were added to 20 ml protoplast isolation medium and gently mixed with 0.25 ml protoplast suspension. The resulting suspension was examined on an epifluorescence photomicroscope (Nikon Eclipse E600 FN, excitation 420-490 nm). The percentage of viable protoplasts was determined from the ratio between fluorescing protoplasts and protoplasts visible under differential interference contrast.

Treatments

Seedlings were grown in MES/Tris buffer (5 mM, pH 6.2) containing 1, 100, or 1000 μM $LaCl_3$ (99.9% heptahydrate, Sigma # L4131, prepared from a 1 M stock solution) or 1 μM ABA for 5 and 24 h. Protoplast suspensions received 0, 1, 100, or 1000 μM $LaCl_3$ in MES/Tris-buffered osmoticum for 5 h. Aliquots of the protoplast suspension were then pelleted and the supernatant removed. About 50 mg of protoplasts from treatments were used for ABA extraction.

Extraction and Quantification of ABA

About 1 g of fresh tissue or about 50 mg of pelleted protoplasts were used for ABA extraction, as described previously (Wang and others 2001). Internal standard (200 ng D_3-ABA) was added to the samples before extraction in a 25-ml flask. Radioactive tracer (20,000 cpm of ^{3}H-ABA, 1.96 TBq/mmol, Amersham Life Science) was added and, after removal of the organic phase, was prepurified on C18 columns (Alltech # 205250, primed with 0.1% acetic acid). The eluant was dried and taken up in 50% MeOH and purified by HPLC (150 × 4.6 mm, ODS, 1 ml/min, 1% acetic acid in 36% MeOH). The retention time for ABA was about 24 min. The fractions with the highest radioactivity were combined, dried in vacuum, methylated with 0.5 ml ethereal diazomethane, dried, and dissolved in 50 µl ethyl acetate. The samples were quantified with HP 6890 GC equipped with a HP 5973 mass selective detector using selected ion monitoring. Chromatography was performed on a capillary column (ZB-5; 30 m × 250 µm × 0.25 µm, Phenomenex) with helium as carrier gas (1.5 ml/min). After 1 min at 160°C the oven temperature was ramped to 280°C at 20°C/min. The retention time of ABA was 5.12 min. The relative abundance of representative fragments for ABA [*m/z* = 190 (authentic ABA) and *m/z* = 193 (deuterated ABA)] was corrected for carryover (D_3-ABA contained 5.4% nondeuterated ABA) and used for quantification. All analyses were repeated three times and data were calculated as mean ± SE in ng/g DW. Statistical analysis was performed using Student's *t* test.

RNA Extraction and Expression Analysis

Total RNA was isolated from control, La^{3+}-, and ABA-treated root tissue using the Plant RNeasy kit (Qiagen, Valencia, CA). Purified RNA from each sample was subjected to DNase treatment and RT-PCR was performed with 1 µg of total RNA (based on absorbance at 260 nm). The resulting cDNA was used to detect the expression of an ABA-related EST (accession No. BQ619400) that was inducible by salt stress (Wang and others 2003) and showed high homology with rice zeaxanthin epoxidase mRNA (ZEP, *E* = 5e-61; NM_001059461). The gene encoding ZEP is similar to *ABA1* in *Arabidopsis* (accession No. NP_851285.1), and we refer to ABA-related EST as putative Zm*ABA1*. The primers (forward: 5′ TCGCAACAAGTG AAGCAAAC 3′; reverse: 5′ ACCCGGAACATAGCCTT CTT 3′) amplified a 320-bp fragment. PCR products were stained with SYBR-Green and checked on a 1.6% agarose gel. The relative intensity of each band was analyzed densitometrically using a gel imaging system (Kodak EDAS 290) and normalized against 18S and 28S rRNA. The experiments were repeated four times.

Results

Root Growth

Lanthanum inhibited elongation and increased the diameter of primary roots of corn (Figure 1). Application of 1 and 100 µM La^{3+} caused 3-5% inhibition, and 1 mM La^{3+} reduced root growth rate by 75% after 24 h compared to controls. The decline in root elongation was noticed within 8-12 h after exposure to 1 and 100 µM La^{3+} (Figure 1A). However, 1 mM La^{3+} reduced growth rate within 8 h, and after 24 h caused radial swelling of root tips and increased the root diameter from 0.8 ± 0.04 mm (control, Figure 1B) to 1.36 ± 0.07 mm (Figure 1C). The swelling was confined to the area between tip and elongation zone and coincided with the promotion of root hairs. Application of 1 µM ABA inhibited root growth by 7%, similar to 100 µM La^{3+} (Figure 1A).

La^{3+} Increased ABA in Intact Roots

Control roots growing in buffer for 5 and 24 h contained 36 ± 4.8 and 23 ± 0.5 ABA (ng g^{-1} FW), respectively. Thus, the endogenous ABA content in untreated roots decreased 35% over time (Figure 2). In the presence of 0.1 or 1 mM La^{3+}, the ABA content increased after 5 h of application by 32% and 87%, respectively. As in controls, longer exposure to La^{3+} corresponded with a decrease in ABA. After 24 h, the lowest La^{3+} concentration (1 µM) had the highest ABA content, similar to untreated controls (Figure 2).

La^{3+} Induced ABA Changes in Protoplasts

Although the number of viable protoplasts decreased over time, the viability of the protoplasts was not affected by La^{3+} treatment (Figure 3). The ABA concentration in La^{3+}-treated protoplasts decreased from 450 ng g^{-1} FW by 50% and 60% in 0.1 and 1 mM La^{3+}-treated protoplasts, respectively (Figure 4). To assess the effect of protoplast preparation, we compared the ABA content in protoplasts with mannitol-treated intact roots. Incubation of roots in 0.6 M mannitol caused cell shrinkage and rapid loss of turgor (data not shown) and a fivefold increase of ABA (200 ng g^{-1} FW) within 5 h compared to the roots that were kept in buffer (Figure 4). Protoplasts (in osmoticum) contained twice the amount of ABA of intact, 5-h osmoticum-treated roots.

ABA-related EST Responds to ABA and La^{3+}

To determine whether ABA changes after La^{3+} treatment resulted from *de novo* synthesis, we measured the

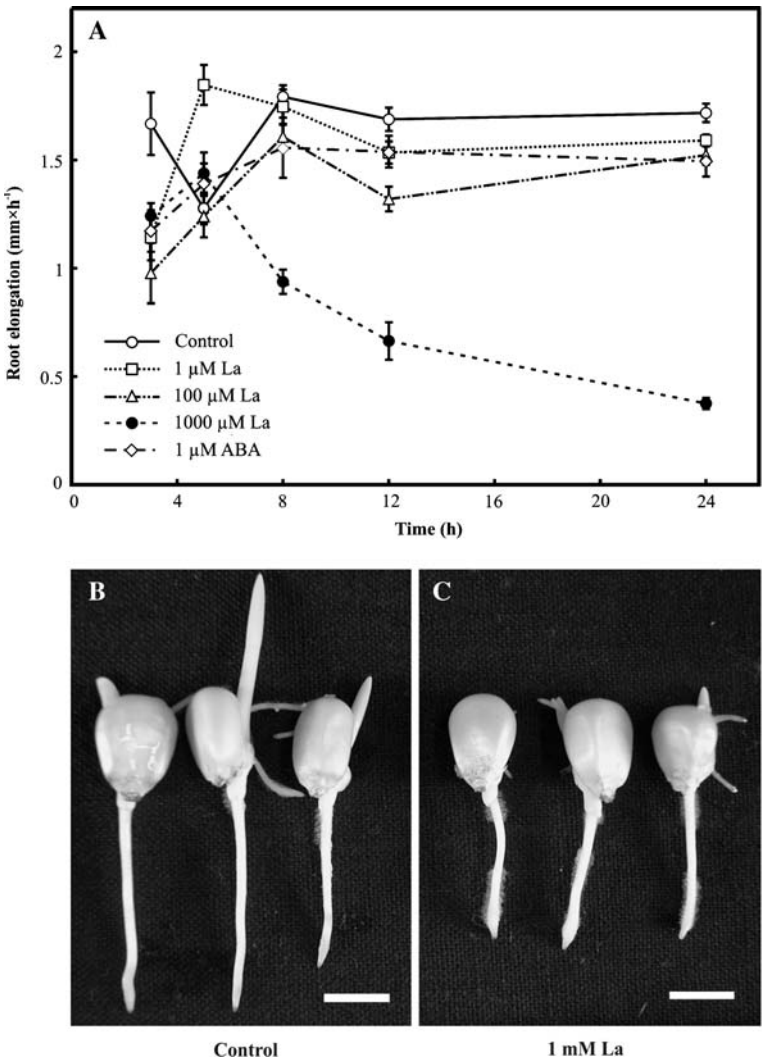

Fig. 1 Effects of La^{3+} on growth of 2-day-old *Zea mays* L. roots show concentration and time-dependent growth inhibition upon exposure to 1, 100, and 1000 μM La^{3+} and 1 μM ABA (**A**). The diameter of root tips increased from 0.8 ± 0.04 mm (control, **B**) to 1.36 ± 0.07 mm and induced root hair formation after exposure to 1 mM La^{3+} for 24 h (**C**). $N = 16 \pm$ SE. Scale bar = 5 mm

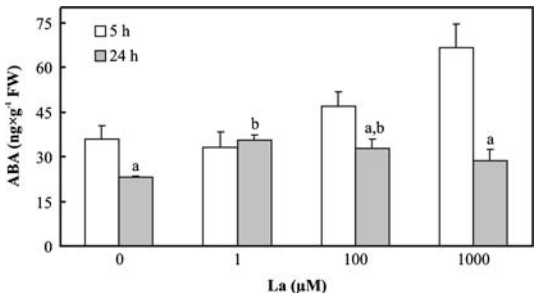

Fig. 2 Effect of La^{3+} on the levels of ABA in roots of maize after exposure for 5 and 24 h. $N = 3 \pm$ SE. The ABA concentration increased after 5 h but decreased after 24 h ($p < 0.05$, a). After 24 h only 1 and 100 μM La^{3+} differed from controls ($p < 0.05$, b)

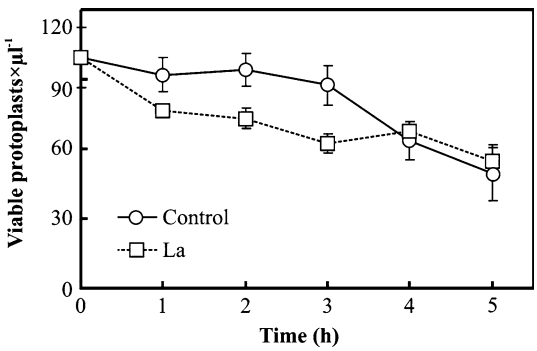

Fig. 3 The viabilities of maize root protoplasts in osmoticum and osmoticum containing 1 mM La^{3+} were similar. $N = 6 \pm$ SE

expression of Zm*ABA1*. Its expression in intact roots was upregulated by La^{3+} and ABA (Figure 5). Exposure to 0.1 and 1 mM La^{3+} for 5 h, the time point that showed visible changes in endogenous ABA level (Figure 2), induced a two- to fourfold change in the Zm*ABA1* expression level, similar to ABA-induced changes (Figure 5).

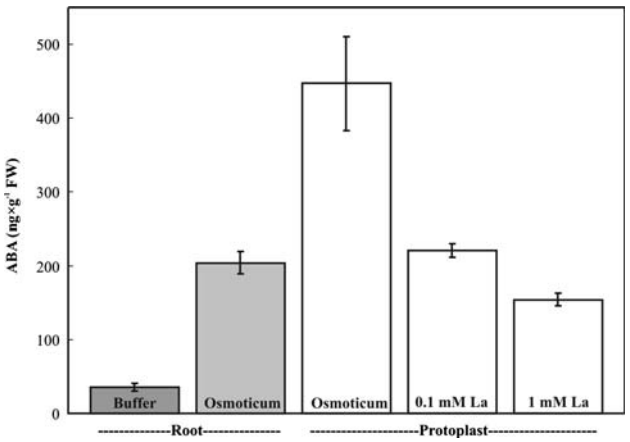

Fig. 4 ABA content in intact roots exposed to MES/Tris buffer or osmoticum was lower than the ABA content in protoplasts that were exposed to the same osmoticum or, additionally, 0.1 or 1 mM La^{3+} for 5 h. $N = 3 \pm SE$

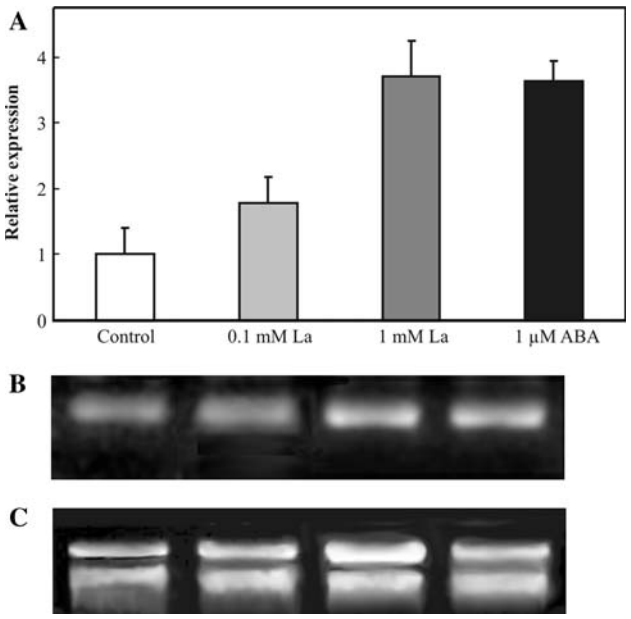

Fig. 5 Relative expression level of putative Zm*ABA1* gene in maize roots exposed to La^{3+} and ABA for 5 h, as analyzed by PCR amplification of cDNA (**A**). The signal was quantified from SYBR-Green-stained agarose gels (**B**). The signal of 18S and 28S rRNA served as reference (**C**). $N = 3 \pm SE$

Discussion

ABA Is Involved in La^{3+}-induced Root Growth Inhibition

The response of plants to stress involves complex physiologic and biochemical processes, including changes in the concentration and distribution of endogenous hormones. ABA is involved in many, if not all, stress responses (Christmann and others 2006; Hasegawa and others 2000; Xie and others 2005), but its precise mode of action is still unclear. We show that La^{3+} inhibits primary root growth in corn (Figure 1) and induces transient ABA accumulation in intact roots (Figure 2). Therefore, the application of La^{3+}as a Ca^{2+} or mechanosensitive channel blocker (Ding and Pickard 1993; Fasano and others 2002) is likely to cause effects beyond the intended use.

The rapid decline of ABA in controls from 36 to 23 ng g^{-1} FW in 18 h (Figure 2) likely results from enhanced water supply after transfer into buffer. This change indicates that roots growing in a nonliquid medium maintain a higher ABA level that probably represents the ABA content required for regular root growth (Ghassemian and others 2000). Even ABA-deficient mutants contain ABA concentrations that are not significantly lower than in the wild type under normal growth conditions (Xiong and Zhu 2003). An increase in ABA in corn roots after short-term La^{3+} exposure (Figure 2) was consistent with an earlier report of La^{3+}-induced ABA in cucumber leaves (Shi and others 2002). However, the subsequent reduction in ABA levels (Figure 2) and the constant growth rate after 12 h (Figure 1A) suggest some adaptation to lanthanum stress. Under prolonged La^{3+} stress (24 h) at high concentrations (>100 μM), La^{3+} uptake into root cells may exert deleterious effects that reduce the capacity to produce ABA. This argument is supported by the observation that the lowest La^{3+} concentration resulted in the highest ABA content after 24 h (Figure 2).

ABA alleviates stress damage through the activation of many stress-response genes for the biosynthesis of osmolytes and LEA-like proteins, which cooperatively improve stress tolerance (Finkelstein and others 2002; Hasegawa and others 2000; Zeevaart and Creelman 1988). Root hair differentiation and inhibition of root elongation are attributed to ethylene (Dolan 2001). Because the ethylene response occurs downstream of the ABA signaling pathway (Ghassemian and others 2000), La^{3+}-induced accumulation of ABA, which is required for the maintenance of primary maize root elongation at low water potential (Saab and others 1990; Sharp and others 2002), also promoted ethylene and root hair growth (Figure 1C). Short-term application of La^{3+} (5 h) led to an increase of ABA, but after 24 h the ABA levels were similar. The increase in Zm*ABA1* (Figure 5) was observed after 5 h and thus is a short-term effect that may be independent of (long-term) ABA-mediated root growth. La^{3+}-induced growth inhibition (Figure 1A, 1 mM La^{3+}) started after 5 h and thus may not be related to the short-term ABA induction in protoplasts (Figure 4). Confirmation of *de novo* synthesis by, for example, labeled precursors might shed light on metabolic changes but would increase the precursor pool and thus promote ABA synthesis independent of the increase of Zm*ABA1*.

La^{3+}, Osmotic Stress, and ABA Accumulation

ABA promotes tolerance to abiotic stresses such as cold, salinity, and drought, all of which induce dehydration (Shinozaki and Yamaguchi-Shinozaki 2000; Xiong and others 2002). The water stress response partially changes root and shoot architecture. Osmotic stress is normally perceived by a reduction in turgor pressure and an increase in cytoplasmic and apoplastic ABA as a result of either *de novo* synthesis or release from organelles (Zeevaart and Creelman 1988). Roots subjected to osmoticum rapidly lost turgor (data not shown) and the ABA content increased about fivefold (Figure 4), indicating an osmotic stress response (Skriver and Mundy 1990). In contrast, osmoticum is necessary to maintain the cell volume of protoplasts. Thus, one could speculate that protoplasts should not be affected by osmotic stress to the extent of intact roots. However, the ABA content in protoplasts was about tenfold that of intact roots (Figure 4). Despite some uncertainty about the weight of the cell wall in a root sample and the interstitial fluid of the protoplast suspension, this observation indicates that in addition to the osmoticum, other factors such as the removal of the cell wall or exposure of or damage to the plasma membrane cause stress. Similar changes in ABA accumulation were observed in other plant tissues under osmotic stress (Bianco-Trichant and others 1993; Bianco-Trichant and Le Page-Degivry 1998; Weiler and others 1982).

Because of the large difference in ABA levels between osmotically stressed but intact roots and protoplasts, it is important to compare the La^{3+} effect on intact roots and protoplasts. La^{3+} reduced the ABA content of protoplasts (Figure 4) but induced ABA in intact roots. It is possible that altered water uptake or changes in the hydraulic conductivity of cell membranes (Hose and others 2000) or fluctuations of intracellular Ca^{2+} contribute to ABA induction (Skriver and Mundy 1990). ABA release may also depend on pH differences (Slovik and Hartung 1992). The intact apoplast (cell wall) may facilitate ABA release possibly via reduced dissociation. Conversely, the removal of the cell wall may lead to an increase in the level of ABA in protoplasts (Bianco-Trinchant and others 1993). La^{3+}, similar to Ca^{2+}, may enhance membrane stability or counteract stress resulting from the loss of the cell wall and thus reduce the ABA content in protoplasts (Figure 4). This relationship could explain the initial increase and subsequent decline of ABA in protoplasts (Figure 2).

Possible Mechanism of La^{3+}-induced ABA Accumulation in Intact Roots

ABA biosynthesis is activated through lipoxygenase activity (Shi and others 2002) and depends upon gene induction (Xiong and Zhu 2003), which may be upregulated by La^{3+}. The *ABA1* gene encodes zeaxanthin epoxidase in *Arabidopsis* (Marin and others 1996) and catalyzes the epoxidation of zeaxanthin and antheraxanthin to violaxanthin (Duckham and others 1991; Barrero and others 2005). Because the *ABA1* gene is commonly expressed in vegetative tissues, we speculate that La^{3+} upregulated ABA results from *ABA1* activation. The transcript level of putative Zm*ABA1* in control roots was low, which corresponds to the low ABA concentration in control roots (Figure 5). However, the expression of Zm*ABA1* increased with the La^{3+} concentration fourfold between 0.1 mM and 1 mM La^{3+}, similar to previous reports (Audran and others 2001; Xiong and Zhu 2003). These authors demonstrated that ABA promotes the expression of its own biosynthetic genes, probably through a Ca^{2+}-dependent phosphoprotein cascade (Xiong and others 2002). However, La^{3+} inhibited calcium-dependent protein kinases, which may be necessary for ABA signaling (Sheen 1996). If La^{3+}-induced signal transduction is similar to that of Ca^{2+} but independent of kinases, as suggested earlier (Shi and others 2002), our data and the results of Shi and others (2002) indicate that Ca^{2+} and La^{3+} actions are at least partially independent of each other. This independence must be considered for studies that use La^{3+} as an Ca^{2+} antagonist.

References

Audran C, Liotenberg S, Gonneau M, North H, Frey A, Tap-Waksman K, Vartanian N, Marion-Poll A (2001) Localisation and expression of zeaxanthin epoxidase mRNA in Arabidopsis in response to drought stress and during seed development. Aust J Plant Phys 28:1161–1173

Barrero JM, Piqueras P, Gonzalez-Guzman M, Serrano R, Rodriguez PL, Ponce MR, Micol JL (2005) A mutational analysis of the *ABA1* gene of *Arabidopsis thaliana* highlights the involvement of ABA in vegetative development. J Exp Bot 56:2071–2083

Bianco-Trinchant J, Guigonis JM, Le Page-Degivry MT (1993) Early release of ABA from cell-walls during rose petal protoplast isolation. J Exp Bot 44:957–962

Bianco-Trinchant J, Le Page-Degivry MT (1998) ABA synthesis in protoplasts of different origin in response to osmotic stress. J Plant Growth Regul 25:135–141

Christmann A, Moes D, Himmelbach A, Yang Y, Tang Y, Grill E (2006) Integration of abscisic acid signalling into plant responses. Plant Biol 8:314–325

Diatloff E, Smith FW, Asher CJ (1995) Rare-earth elements and plant-growth 1. Effects of lanthanum and cerium on root elongation of corn and mungbean. J Plant Nutr 18:1963–1976

Ding JP, Pickard BG (1993) Mechanosensory calcium-selective cation channels in epidermal cells. Plant J 3:83–110

Dolan L (2001) The role of ethylene in root hair growth in *Arabidopsis*. J Plant Nut Soil Sci 164:141–145

dos Remedios CG (1981) Lanthanide ion probes of calcium-binding sites on cellular membranes. Cell Calcium 2:29–51

Duckham SC, Linforth RST, Taylor IB (1991) Abscisic-acid-deficient mutants at the *aba* gene locus of *Arabidopsis thaliana* are impaired in the epoxidation of zeaxanthin. Plant Cell Environ 14:601–606

Fasano JM, Massa GD, Gilroy S (2002) Ionic signaling in plant responses to gravity and touch. J Plant Growth Regul 21:71–88

Finkelstein RR, Gampala SSL, Rock CD (2002) Abscisic acid signaling in seeds and seedlings. Plant Cell 14:S15–S45

Gampala SSL, Hagenbeek D, Rock CD (2001) Functional interactions of lanthanum and phospholipase D with the abscisic acid signaling effecters VP1 and ABI1-1 in rice protoplasts. J Biol Chem 27:69855–69860

Gelli A, Blumwald E (1997) Hyperpolarization-activated Ca^{2+}-permeable channels in the plasma-membrane of tomato cells. J Membr Biol 155:35–45

Ghassemian M, Nambara E, Cutler S, Kawaide H, Kamiya Y, Mccourt P (2000) Regulation of abscisic acid signaling by the ethylene response pathway in *Arabidopsis*. Plant Cell 12:1117–1126

Hagenbeek D, Quatrano RS, Rock CD (2000) Trivalent ions activate abscisic acid-inducible promoters through an ABI1-dependent pathway in rice protoplasts. Plant Physiol 123:1553–1560

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. Ann Rev Plant Physiol Plant Mol Biol 51:463–499

He YW, Loh CS (2000) Cerium and lanthanum promote floral initiation and reproductive growth of *Arabidopsis thaliana*. Plant Sci 159:117–124

Hose E, Steudle E, Hartung W (2000) Abscisic acid and hydraulic conductivity of maize roots: a study using cell- and root-pressure probes. Planta 211:874–882

Knight H, Trewavas AJ, Knight MR (1997) Calcium signalling in *Arabidopsis thaliana* responding to drought and salinity. Plant J 2:1067–1078

Lehmann H, Stelzer R, Holzamer S, Kunz U, Gierth M (2000) Analytical electron microscopical investigations on the apoplastic pathways of lanthanum transport in barley roots. Planta 211:816–822

Liu M, Hasenstein KH (2005) La^{3+} uptake and its effect on the cytoskeleton in root protoplasts of *Zea mays* L. Planta 220:658–666

Loveys BR, Robinson SP (1987) Abscisic acid synthesis and metabolism in barley leaves and protoplasts. Plant Sci 49:23–30

Marin E, Nussaume L, Quesada A, Gonneau M, Sotta B, Hugueney P, Frey A, Marionpoll A (1996) Molecular identification of zeaxanthin epoxidase of *Nicotiana plumbaginifolia*, a gene involved in abscisic acid biosynthesis and corresponding to the ABA locus of *Arabidopsis thaliana*. EMBO J 15:2331–2342

Martin RB, Richardson FS (1979) Lanthanides as probes for calcium in biological systems. Q Rev Biophys 12:181–209

Quiquampoix H, Ratcliffe RG, Ratkovic S, Vucinic Z (1990) ^{1}H and ^{31}P NMR investigation of gadolinium uptake in maize roots. J Inorg Biochem 38:265–275

Rock CD, Quatrano RS (1996) Lanthanide ions are agonists of transient gene expression in rice protoplasts and act in synergy with ABA to increase *Em* gene expression. Plant Cell Rep 15:371–376

Saab IN, Sharp RE, Pritchard J, Voetberg GS (1990) Increased endogenous abscisic-acid maintains primary root growth and inhibits shoot growth of maize seedlings at low water potentials. Plant Physiol 93:1329–1336

Sharp RE, Lenoble ME (2002) ABA, ethylene and the control of shoot and root growth under water stress. J Exp Bot 53:33–37

Sheen J (1996) Ca^{2+}-dependent protein kinases and stress signal transduction in plants. Science 274:1900–1902

Sheen J (2001) Signal transduction in maize and Arabidopsis mesophyll protoplasts. Plant Physiol 127:1466–1475

Shi P, Zeng F, Song W, Zhang M, Deng R (2002) Effects of calcium and lanthanum on ABA biosynthesis in cucumber leaves. Rus J Plant Physiol 49:696–699

Shinozaki K, Yamaguchi-Shinozaki K (2000) Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. Cur Opin Plant Biol 3:217–223

Skriver K, Mundy J (1990) Gene expression in response to abscisic acid and osmotic stress. Plant Cell 2:503–512

Slovik S, Hartung W (1992) Compartmental distribution and redistribution of abscisic-acid in intact leaves. III: Analysis of the stress-signal chain. Planta 187:37–47

Thomson WW, Platt KA, Campbell N (1973) Use of lanthanum to delineate apoplastic continuum in plants. Cytobios 8:57–62

Tyler G (2005) Rare earth elements in soil and plant systems - A review. Plant Soil 267:191–206

Van Steveninck RFM, van Steveninck ME, Chescoe D (1976) Intracellular binding of lanthanum in root tips of barley (*Hordeum vulgare*). Protoplasma 90:89–97

Wang H, Miyazaki S, Kawai K, Deyholos M, Galbraith DW, Bohnert HJ (2003) Temporal progression of gene expression responses to salt shock in maize roots. Plant Mol Biol 52:873–891

Wang YY, Mopper S, Hasenstein KH (2001) Effects of salinity on endogenous ABA, IAA, JA and SA in *Iris hexagona*. J Chem Ecol 27:327–342

Weiler EW, Schnabl H, Hornberg C (1982) Stress-related levels of abscisic-acid in guard-cell protoplasts of *Vicia faba* L. Planta 154:24–28

Xie Z, Ruas P, Shen QJ (2005) Regulatory networks of the phytohormone abscisic acid. Vitam Horm 72:235–269

Xiong LM, Zhu JK (2003) Regulation of abscisic acid biosynthesis. Plant Physiol 133:29–36

Xiong LM, Schumaker KS, Zhu JK (2002) Cell signaling during cold, drought, and salt stress. Plant Cell 14:S165–S183

Zeevaart JAD, Creelman RA (1988) Metabolism and physiology of abscisic acid. Ann Rev Plant Physiol Plant Mol Biol 39:439–473