A Novel Function of Abscisic Acid in the Regulation of Rice (Oryza sativa L.) Root Growth and Development

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Plant roots retain developmental plasticity and respond to environmental stresses or exogenous plant growth regulators by undergoing profound morphological and physiological alteration. In this study, we investigated the effects of exogenous ABA on root growth and development in Taichung native 1 (TN1) rice. Exogenous application of 10 µM ABA leads to swelling, root hair formation and initiation of lateral root primodia in the tips of young, seminal rice roots. Cortex cells increased in size and were irregularly shaped. ABA treatment significantly increased 2, 3, 5-triphenyl tetrazolium chloride (TTC) reductase ability in the root tips and the exudation rate of xylem sap. In addition, the K⁺ ion content in xylem sap increased nearly 2-fold, but not that of Ca²⁺ or Mg²⁺. Analysis of proteins expressed in the root tips identified several ABA-induced or -repressed proteins, including actin depolymerization factor (ADF), late embryo abundant protein (LEA), putative steroid membrane-binding protein, ferredoxin thionine reductase and calcium-binding protein. The effects of ABA on root morphogenesis change were Ca²⁺ dependent and required the participation of calmodulin and de novo protein synthesis. A model is presented that illustrates how ABA acts through a potential cellular and signal transduction mechanism to induce morphological and physiological changes in rice roots.

Keywords: Abscisic acid (ABA) — Actin depolymerization factor (ADF) — Lateral root — Oryza sativa L. — Proteomics.

Abbreviations: ADF, actin depolymerization factor; DMSO dimethylsulfoxide; IPG, immobilized pH gradient; LC/MS/MS, liquid chromatography tandem mass spectrometry; LEA, late embryonic abundant protein; TN1, Taichung native 1; TTC, 2,3,5-triphenyl tetrazolium chloride.

Introduction

Root systems are crucial for plant growth: they acquire water and nutrients and provide anchorage. The proper establishment of basic root architecture in response to environmental change is important for many agronomic traits, including lodging tolerance, drought tolerance and the final crop yield (Lynch 1995, Teo et al. 1995). Variation in root system architecture, including lateral root branching and root hair density, can greatly change crop plant vigor by affecting water use efficiency and nutrient extraction under different growing conditions (Malamy and Benfey 1997). Root system architecture is determined by an endogenous genetic program and external factors, such as the distribution of nutrients in the soil and environmental stresses. Investigation of how roots detect levels of water and nutrients in the soil and change their architecture adaptivity is not only of interest for the study of root growth and development but also for the practice of agronomical significance.

The root system of rice (Oryza sativa L.) consists of seminal roots, adventitious roots and lateral roots. For all rice root types, the growing parts can be longitudinally divided into the root cap, apical meristem, the elongation zone and the maturation zone. Elongation of root hair growth is calcium dependent. Once a root hair has been initiated in Arabidopsis, high cytosolic Ca²⁺ is found at the root hair apex and it is correlated with the subsequent growth of the root hair tip. Treatment with the Ca²⁺ channel blocker verapamil decreased levels of cytosolic Ca²⁺ and led to the inhibition of root hair growth (Bibikova et al. 1997, Wymer et al. 1997, Gilroy and Jones 2000).

External and internal factors, including nutrients such as sugars, ammonium, nitrite and phytohormones, can regulate the initiation and growth of plant roots. Many plant species, when exposed to a locally concentrated source of NO₃⁻, NH₄⁺ or low fertility soil deficient in mobilized nutrients such as P and Fe, respond by proliferating their lateral roots to form a proteoid, a cluster of root-like structures (Neumann and Martinoia 2002). In Arabidopsis, under restrict NO₃⁻ supply, a localized stimulatory effect on stimulation of lateral root elongation was noted. However, with the presence of uniformly

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Abscisic acid and root growth and development in rice

High NO$_3^-$, a systemic inhibitory effect that blocked activation of the lateral root meristem was observed just after its emergence from the primary root. In addition, a local NO$_3^-$ supply clearly stimulated lateral root growth in rice (Wang et al. 2002). Several other plant hormones, including auxin, ethylene, ABA and jasmonic acid, participate in the regulation of plant root growth and development (Spollen et al. 2000, Wang et al. 2002, Ma et al. 2003, Smet et al. 2003). Interactions between plant hormones add further complexity to the study of root growth and development in a heterogeneous soil environment.

ABA is considered a plant stress hormone. It controls plant development and growth, including embryo development, seed dormancy, transpiration and adaptation to environmental stresses such as cold, salt and drought (Zeevaart and Creelman 1988, Campalans et al. 1999). In plants experiencing drought, ABA is important in root-to-shoot signaling in stomatal closure, slowing shoot growth and maintaining primary root elongation. Typically, exogenously applied ABA inhibited shoot and root growth in well-watered plants. However, at low water potentials, accumulation of endogenous ABA restricted ethylene production in response to compacted soil and maintained continuous root elongation (Sarquis et al. 1991, Roberts et al. 2002, Sharp and LeNoble 2002). ABA not only affects plant primary root elongation but also regulates lateral root formation. Two recent studies demonstrated the importance of ABA in mediating the inhibitory effect of high nitrate levels on lateral root formation in Arabidopsis (Signora et al. 2001, Smet et al. 2003). The high-nitrate-induced lateral root inhibition was significantly reduced in two ABA-insensitive mutants, $abi4$ and $abi5$. In addition, exogenous ABA induced

![Fig. 1](https://academic.oup.com/pcp/article-abstract/47/1/1/1867452)
lateral root inhibition as did high NO$_3^-$, and this pathway is auxin independent. Determining the mechanism by which ABA regulates root development remains a major challenge.

Previously, we demonstrated that the increase of internal ABA levels is closely related to chilling tolerance of rice seedlings, and exogenously applied ABA can ameliorate chilling injury in Taichung native 1 (TN1) rice exposed to cold (Lee et al. 1993, Lee et al. 1995). Meanwhile, we observed that exogenous ABA can affect the growth and development of rice roots. Therefore, in this study, we focused on characterization of the development, anatomical and physiological responses of TN1 rice roots to exogenous applications of ABA. To investigate the cellular mechanism and signal transduction pathway by which ABA induces lateral root formation, we conducted a proteomic survey of lateral root tip protein expression and a pharmalogical study using different inhibitors. Lastly, we propose a putative scheme for how cells that receive ABA transduce its signal to affect root tip swelling, root hair formation and lateral root production in TN1 rice.

**Results**

**ABA induced time course-dependent morphological changes in TN1 rice root tips**

To test whether exogenously applied ABA affects rice root growth and development, the morphology of untreated TN1 rice seedling roots was compared with that of roots from seedlings grown with 10 µM ABA for 10 d. This level of ABA effectively ameliorates chilling damage in rice seedlings of this age (Lee et al. 1993, Lee et al. 1995). ABA in the growth medium induces a range of morphological changes in TN1 roots (Fig. 1). The changes occurred in four stages. In stage I,
Abscisic acid and root growth and development in rice

From zero to about 24 h after the start of ABA treatment, the root tips directly behind the root cap swelled and formed ellipsoidal dilatations with root hairs on the surface. These morphological changes were confined mostly to young seminal roots with exuberant differentiation abilities (Fig 1a–c). No root hairs formed on the root tips of untreated seedlings during the first 24 h.

During stage II, from about 24 to 48 h after the addition of exogenous ABA, the swollen seminal root tips continued to lengthen and primary lateral root primordia emerged near or on the dilatations away from the distal end (Fig. 1d–f). During stage III, typically after 3–4 d of ABA treatment, the primary lateral root primordia started to elongate, swelled and formed new root hairs on the surface like previous seminal roots (Fig. 1g–l). During stage IV, which resulted from continuous treatment with ABA for 5–6 d, secondary lateral root primordia were initiated on the primary lateral rootlets growing from the swollen, seminal root tips. The secondary lateral roots were short and small, and did not continue to swell. In this stage, the roots formed bunchy, broom-like structures (Fig. 1m–o).

To assess the long-term effects of ABA on the growth and development of TN1 rice roots, seedlings were grown for 2 weeks in medium with 10 µM ABA. Treated seedlings formed a much larger number of short lateral roots in the cluster root zone than control (CK) seedlings (Fig. 1p). When treated with ABA for 3–4 weeks, roots formed discontinuous, repeated units with a branch-like structure whenever ABA was added. These structures were similar to cluster roots (proteoid roots) (Fig. 1q, r).

ABA treatment caused root tip cells to enlarge transversely and longitudinally

ABA-induced swelling of root tips could be caused by an increase in cell size or number. To understand better how root tip cells respond to ABA, the development of root tip cells from control and ABA-treated seedlings was analyzed by histomicroscopy. In cross-sections of control seedling root tips, epidermal and exodermal cells were closely adjoined and the boundary between them was indiscernible. The cortical cells were aligned and formed regular ellipses (Fig. 2a). Cells in the vascular cylinder were immature and the secondary cell wall was not visible. In contrast, cells in ABA-treated root tips were irregularly shaped and less organized. During treatment with ABA over 3 d (Fig. 2b–d), root cortical cells expanded isodiametrically (compare with Fig. 2a). After 1 d of ABA treatment, epidermal cells had increased in size and become irregularly shaped, but not to the same degree exhibited by cortical cells (Fig. 2b). With continued exposure to ABA, epidermal and exodermal cells continued to enlarge, and clear boundaries formed between them. Sclerenchyma cells changed little in size or shape (Fig. 2c, d). ABA treatment of TN1 roots did not change the shape of vascular cylinder cells. Some of these cells underwent secondary growth after 1 d of ABA treatment. With 2–3 d of exogenous ABA treatment, they reached maturity and formed early metaxylem. Formation of lateral roots from metaxylem poles can be seen easily in transverse sections (Fig. 2c, d).

The same changes in cell size and organization were observed in longitudinal sections. In CK seedling root tips, cortical cells appeared as orderly arranged rectangles (Fig. 2e). In ABA-treated seedlings, root tip cortical cells expanded in size, and became irregularly shaped (Fig. 2f–h). In addition, in ABA-treated roots, vessel elements and tracheids formed in the vascular cylinder (Fig. 2g, h). Thus, ABA caused root tip swelling by increasing cell size rather than accelerating cell division to increase cell number.

ABA enhanced 2,3,5-triphenyl tetrazolium chloride (TTC) reductase activity, elevated xylem sap exudation rate and promoted uptake of potassium ions in roots

Under adverse conditions, ABA can alter plant root water permeability, regulate water flow and increase plant vitality. In
Abscisic acid and root growth and development in rice

Exogenous ABA can increase the root exudation rate ($J_v$) and hydraulic conductivity ($L_p$) (Javot and Maurel 2002). Therefore, experiments were conducted to determine how ABA-induced morphological changes in root architecture affect the physiological responses of rice roots.

First, the vitality of ABA-treated rootlets was monitored with the TTC staining procedure. The TTC test has been used as a qualitative indicator of cell vitality and is based on the principle that all living tissue contains active dehydrogenase enzymes that catalyze chemical reductions (Steponkus and Lanphear 1967). TTC reductase activity was measured in TN1 roots from seedlings treated with ABA for 1, 3, 5 or 7 d. The area showing the greatest activity (which appears red) was the swollen root tip, especially the newly initiated root primordia (data not shown). TTC reductase activity in CK seedlings remained almost unchanged, but in ABA-treated roots it increased 2- to 4-fold over 7 d (Fig. 3a). The vitality of ABA-treated roots was greater than that of seedlings grown in normal culture medium.

Sunflower, barley, sorghum and maize, exogenous ABA can increase the root exudation rate ($J_v$) and hydraulic conductivity ($L_p$) (Javot and Maurel 2002). Therefore, experiments were conducted to determine how ABA-induced morphological changes in root architecture affect the physiological responses of rice roots.

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Secondly, because ABA affects the regulation of water flow, its effect on the water uptake of rice roots was measured. Within 24 h of the start of ABA treatment, treated seedlings exhibited a marked increase in xylem sap exudation volume but CK seedlings did not (Fig. 3b). By the fourth hour of collection, the exudation volume of ABA-treated seedlings was 2.3-fold higher than that of CK seedlings.

Thirdly, the acceleration of water uptake caused by ABA helps plant roots endure abiotic stresses by preventing water loss. Increasing water uptake could be achieved by changing
Table 1 Identification of ABA-induced or repressed proteins from two dimensional gels using LC/MS/MS method

<table>
<thead>
<tr>
<th>Spot no.</th>
<th>Theoretical/observed M&lt;sub&gt;r&lt;/sub&gt;</th>
<th>% of sequence covered</th>
<th>Accession no.</th>
<th>Protein identified species</th>
<th>Homologous protein</th>
<th>Amino acid sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ra</td>
<td>42.1/44.4</td>
<td>21</td>
<td>CAD79700</td>
<td>Oryza sativa</td>
<td>G-3-P dehydrogenase</td>
<td>M(21) CAD79700 Oryza sativa G-3-P dehydrogenase</td>
</tr>
<tr>
<td>Rb</td>
<td>42.1/44.4</td>
<td>31</td>
<td>CAD79700</td>
<td>Oryza sativa</td>
<td>G-3-P dehydrogenase</td>
<td>M(21) CAD79700 Oryza sativa G-3-P dehydrogenase</td>
</tr>
<tr>
<td>RT16</td>
<td>24.6/42.9</td>
<td>25</td>
<td>AAG13629</td>
<td>Oryza sativa</td>
<td>Putative steroid membrane-binding protein</td>
<td>M(25) AAG13629 Oryza sativa</td>
</tr>
<tr>
<td>R4</td>
<td>16.0/18.7</td>
<td>44</td>
<td>AAG28460</td>
<td>Thino- pyr rum elongatum</td>
<td>Actin-depolymerizing factor</td>
<td>M(44) AAG28460 Thino- pyr rum elongatum</td>
</tr>
<tr>
<td>R5</td>
<td>16.0/17.7</td>
<td>44</td>
<td>AAG28460</td>
<td>Thino- pyr rum elongatum</td>
<td>Actin-depolymerizing factor</td>
<td>M(44) AAG28460 Thino- pyr rum elongatum</td>
</tr>
<tr>
<td>R6</td>
<td>16.2/12.8</td>
<td>32</td>
<td>BAB32715</td>
<td>Oryza sativa</td>
<td>LEA protein</td>
<td>M(32) BAB32715 Oryza sativa</td>
</tr>
<tr>
<td>RT8</td>
<td>68.6/75.1</td>
<td>12</td>
<td>JC4395</td>
<td>Oryza sativa</td>
<td>Ferredoxin-nitrite reductase</td>
<td>M(12) JC4395 Oryza sativa</td>
</tr>
<tr>
<td>R2</td>
<td>33.5/30.6</td>
<td>18</td>
<td>CAED1785.1</td>
<td>Oryza sativa</td>
<td>Calcium-binding protein</td>
<td>M(18) CAED1785.1 Oryza sativa</td>
</tr>
<tr>
<td>R1</td>
<td>46.1/42.1</td>
<td>11</td>
<td>AAL7132.1</td>
<td>Oryza sativa</td>
<td>Cationic peroxidase</td>
<td>M(11) AAL7132.1 Oryza sativa</td>
</tr>
</tbody>
</table>

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Abscisic acid and root growth and development in rice
ABA-responsive ones. Proteins were extracted from untreated tip to eliminate most other abundant root proteins and enrich µ with 10 root tips of TN1 rice seedlings. TN1 rice seedlings were treated conducted a comparative proteomic analysis with intact roots and insight into this process.

transduction pathway and the effect of ABA on the morphological features of roots remains to be established. Thus, both proteomic and pharmalogical studies were conducted to gain insight into this process.

To identify the response of root proteins to ABA, we conducted a comparative proteomic analysis with intact roots and root tips of TN1 rice seedlings. TN1 rice seedlings were treated with 10 µM ABA for 24 h. Roots were cut 0.5 cm from the root tip to eliminate most other abundant root proteins and enrich ABA-responsive ones. Proteins were extracted from untreated and ABA-treated seminal roots. Extracts analyzed with 2D PAGE marker software contained >1,000 proteins with a pH range of 4–7 and a size range of 10–120 kDa. ABA induced significant increases in 15 proteins and repressed one protein, spot RT16 (42.9 kDa, pl 4.18) (Fig. 5, 6). The proteins induced by ABA were: Ra (44.4 kDa, pl 7.12), Rb (44.4 kDa, pl 7.81), R2 (47.2 kDa, pl 4.77), R3 (44.4 kDa, pl 5.35), R4 (18.7 kDa, pl 4.4), R5 (17.7 kDa, pl 4.52), R6 (12.8 kDa, pl 4.68), R7 (18.7 kDa, pl 5.82), RT9 (44.4 kDa, pl 5.75), RT10 (55.3 kDa, pl 5.53), RT11 (28.6 kDa, pl 6.41), RT12 (26.8 kDa, pl 5.54), RT13 (17.7 kDa, pl 4.95), RT14 (17.9 kDa, pl 6.18) and RT15 (10.9 kDa, pl 5.33) (R, proteins from intact roots; RT, proteins from root tips). Eight ABA-induced proteins and one ABA-repressed protein were characterized further with liquid chromatography/tandem mass spectrometry (LC/MS/MS) (Table 1). The peptide sequences of these proteins exhibited 11–44% homology to those of known proteins. The protein spots had high amino acid identity with other proteins as follows: glyceraldehyde-3-phosphate dehydrogenase (Ra and Rb), calcium-binding protein (R2), actin depolymerization factor (ADF) (R4 and R5), late embryo abundant protein (LEA) (R7), ferredoxin-nitrite reductase (RT8) and putative steroid membrane-binding protein (RT16). Two adjacent protein spots, R4 and R5, both were identified as ADF, and were renamed ADF-1 and ADF-2, respectively. Interestingly, kinetic analysis of ADF expression revealed that ADF-1 was induced after 1 h of ABA treatment, but ADF-2 first appeared after 3 h of ABA treatment. ADF-1 and ADF-2 expression increased with prolonged ABA treatment and reached a plateau in about 24 h (Fig. 7).

ABA-induced lateral root formation is calcium and calmodulin-dependent and requires de novo protein synthesis

Calcium participates in the regulation of root tip and root hair growth (Bibikova et al. 1997) and may regulate lateral root formation. To determine the role of Ca2+ in the subsequent signal transduction events in ABA-induced root morphological changes in TN1 roots, eight Ca2+-related inhibitors were used as probes to dissect this process.

EGTA, BAPTA, LaCl3 and GdCl3 are extracellular Ca2+-influx chelators or membrane blockers. Ruthenium red, caffeine and LiCl, are intracellular Ca2+ release blockers and primarily affect mitochondria and endoplasmic reticulum. In TN1 rice, all chelators and blockers completely inhibited the induction effect of ABA on root tip swelling, root hair growth and lateral root formation (Fig. 8a, b, f; Table 2). Interestingly, a Ca2+ channel inhibitor antagonist, A23187, delayed root tip swelling but strongly promoted lateral root emergence in the presence of ABA (Fig. 8c). Thus, after roots received a signal from the exogenous ABA, the Ca2+ concentration in the cytoplasm increased. The Ca2+ is derived from both intra- and extracellular sources and it is essential for induction of lateral root formation. Another signal transduction pathway related to Ca2+ occurs through the effect of a downstream component, calmodulin. To probe the importance of calmodulin in ABA-
induced root change, the inhibitor W7 was used for this experiment. W7 completely prevented ABA from affecting the root morphology of TN1 seedlings (Fig. 8d). Finally, cycloheximide was used to determine whether the process for ABA-induced lateral root formation requires de novo protein synthesis. Cycloheximide completely inhibited ABA-induced root tip swelling (Fig. 8e). Therefore, in addition to Ca\textsuperscript{2+}, calmodulin participates in ABA-induced lateral root formation; this process requires the de novo synthesis of an unknown protein(s).

**Discussion**

Our objectives are to understand the role of ABA in the regulation of rice root growth and development. Therefore, we characterized the morphological changes, physiological responses, protein expression profiles and putative signal transduction events in TN1 rice roots treated with ABA.

**Table 2** Effect of inhibitors on ABA-induced responses: root tip swelling, root hair formation and the emergence of lateral roots near the root tip

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Pharmacological action</th>
<th>Concentration used</th>
<th>Response (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGTA</td>
<td>Ca\textsuperscript{2+} chelator</td>
<td>1 mM, 10 mM, 25 mM (^b)</td>
<td>Fully inhibited (25 mM)</td>
</tr>
<tr>
<td>BAPTA</td>
<td>Ca\textsuperscript{2+} chelator</td>
<td>1 mM, 5 mM, 10 mM (^b)</td>
<td>Fully inhibited (5 mM)</td>
</tr>
<tr>
<td>LaCl\textsubscript{3}</td>
<td>Plasma membrane Ca\textsuperscript{2+} channel blocker</td>
<td>0.05 mM, 0.5 mM, 5 mM (^b)</td>
<td>Fully inhibited (0.5 mM)</td>
</tr>
<tr>
<td>LiCl\textsubscript{3}</td>
<td>Intracellular Ca\textsuperscript{2+} blocker</td>
<td>1 mM, 5 mM, 10 mM (^b)</td>
<td>Partially inhibited (5 mM)</td>
</tr>
<tr>
<td>GdCl\textsubscript{3}</td>
<td>Plasma membrane Ca\textsuperscript{2+} channel blocker</td>
<td>0.05 mM, 0.5 mM, 5 mM (^b)</td>
<td>Fully inhibited (0.5 mM)</td>
</tr>
<tr>
<td>A23187</td>
<td>Ca\textsuperscript{2+} ionophore</td>
<td>10 (\mu)M, 50 (\mu)M (^b)</td>
<td>Root tip swelling was delayed, but the emergence of lateral roots was promoted</td>
</tr>
<tr>
<td>Ruthenium red</td>
<td>Mitochondrial Ca\textsuperscript{2+} channel blocker</td>
<td>5 (\mu)M, 10 (\mu)M, 20 (\mu)M (^b)</td>
<td>Fully inhibited (10 (\mu)M)</td>
</tr>
<tr>
<td>Caffeine</td>
<td>cADP-ribose gated ER Ca\textsuperscript{2+} channel blocker</td>
<td>1 mM, 5 mM, 10 mM (^b)</td>
<td>Fully inhibited (5 mM)</td>
</tr>
<tr>
<td>W-7</td>
<td>Calmodulin competitor</td>
<td>0.05 mM, 0.1 mM, 0.2 mM (^b)</td>
<td>Fully inhibited (0.1 mM)</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>De novo protein synthesis inhibitor</td>
<td>0.0005 mM, 0.01 mM, 0.05 mM (^b), 0.1 mM (^b), 0.5 mM</td>
<td>Fully inhibited (0.05 mM)</td>
</tr>
</tbody>
</table>

\(^a\) Indicated root tip swelling, root hair formation and the emergence of lateral roots near the root.

\(^b\) Indicated cytotoxicity at this concentration.
Root tip swelling and lateral root formation in rice seedlings are a novel function of ABA

In TN1 root tips, exogenous ABA induces formation of lateral root primordia and promotes the maturation of cells in the epidermis and vascular cylinder as root hairs and lateral roots appeared in the maturation zone. These anatomical observations suggest that ABA can transmit a signal to these cells, either directly or indirectly, to alter cell fates and increase formation of lateral roots. Interestingly, long-term exposure to ABA caused rice roots to form bunchy, broom-like structures similar to cluster roots. The same structures are formed by the roots of ABA-treated sorghum seedlings (3 weeks old) and the excised root segments of rice cultivar TCS10 grown in vitro (unpublished results). The ABA-induced root branching observed here is quite similar to the cluster root-like structures that appear in rape seed (Brassicaceae), tomato (Solanaceae) and spinach (Chenopodiaceae) suffering phosphate deprivation (Foehse and Jungk 1983). In castor beans deprived of phosphate, ABA synthesis in the roots and transport in the xylem were highly elevated (Jeschke et al. 1997). Extra ABA in the roots of phosphate-deficient plants may contribute to the increased growth of root hairs.

Elongation of seminal roots and lateral roots, and the initiation of lateral roots, are stimulated by water deficiency (Yang et al. 2003, Yang et al. 2004). Increased seminal root growth results primarily from elongation of cortical cells in the elongation zone. Comprehensive gene expression analysis of water-deficient rice seedlings by cDNA amplified fragment length polymorphism (AFLP) identified a gene, 9-cis-epoxycarotenoid dioxygenase (NCED1), which is involved in ABA biosynthesis. A high level of endogenous ABA was thought to be required for elongation of rice seminal roots. However, in this study, we showed that exogenous application of ABA to TN1 causes root tip swelling and induces lateral root formation, primarily by triggering vascular differentiation in the elongation zone. ABA not only plays a role in plant growth responses to water deficit but may also be essential for plant growth responses to environmental variation.

ABA elevated the vitality of TN1 roots and promoted the uptake of water and potassium ions

Plants synthesize ABA in the roots in response to abiotic stresses. It increases transport through the xylem but not the phloem. Accumulation of endogenous ABA may be correlated with the physiological adjustments plants make to adverse environments. In this study, the effect of exogenous ABA on TTC reductase activity supports this interpretation. Stain was restricted to swollen rice root tips and lateral root primordia, which indicates that ABA enhanced root tip respiration. Exogenously applied ABA greatly increases ATP generation in rice root cells, providing protection from harsh growing conditions. In TN1 seedlings, exogenous ABA increases the exudation rate of xylem sap, possibly by causing swelling and branching of root tips, which expand their surface area, or by altering the water permeability of cell membranes. In plant cells, water flow is regulated by the different aquaporin isoforms (Javot and Maurel 2002). How ABA mediates up-regulation of water transport in TN1 rice, possibly through modulation of aquaporin isofrom expression, requires further study. ABA promoted the uptake of K⁺ ions in xylem sap, but did not affect the amount of Ca²⁺ and Mg²⁺ ions. This is consistent with results from a study on maize in which ABA pre-treatment increased K⁺ ion channel activity in stele responsible for the time-dependent inward current (more K⁺ ions are transported into xylem) (Roberts 1998). K⁺ is the major osmotically active cation responsible for the maintenance of root cell turgor and expansion. Potassium ions also participate in the activation of a number of enzymes, especially those involved in photosynthesis and respiration. The xylem sap of ABA-treated, TN1 seedling roots contains high levels of K⁺. Elevated K⁺ levels strongly increase root vitality during adverse conditions.

The effects of ABA on root tip swelling and lateral root formation involve elevation of cytosolic Ca²⁺, the participation of calmodulin and de novo protein synthesis

The change in cytosolic calcium mediated by ABA is considered as a secondary messenger that triggers a cellular response. In this study, we showed that a variety of different extracellular (e.g. EGTA, BAPTA, LaCl₂ and GdCl₂) and intracellular (e.g. ruthenium red and caffeine) calcium inhibitors blocked the effect of ABA on rice root tip swelling and lateral root formation. Elevated Ca²⁺ in the cytosol, derived from both intra- and extracellular sources, is essential for the initiation of downstream signal propagation by ABA. LiCl, which blocks the phosphoinositide cascade, only partially blocked root tip swelling. Other calmodulin and protein synthesis inhibitors also blocked ABA-induced lateral root formation. Thus, activated Ca²⁺-calmodulin complexes and new protein synthesis seem to activate downstream signaling components that finally lead to the observed changes in root morphology. The proteomic survey showed that exogenous application of ABA to TN1 seedling roots led to de novo synthesis of specific proteins. Of these, glyceraldehyde-3-phosphate dehydrogenase, LEA and cationic peroxidase protein may be involved in tolerance to energy deprivation, cellular dehydration, oxidative stress upon desiccation, salt stress and chilling. ABA also increases expression of ferredoxin-nitrite reductase, which participates in nitrogen fixation. The conversion of nitrite to ammonia and then glutamine may provide amino acids for protein synthesis at low temperatures. In addition, two ADF proteins that have the same amino acid sequences, but may be phosphorylated or dephosphorylated, were differentially induced by ABA. These proteins occur in TN1 root tips subjected to chilling. Interestingly, drought stress also induced ADF synthesis in rice, indicating that the rate of actin turnover could be increased (Salekdeh et al. 2002, Yang et al. 2003). In plants, cold acclimation is associated with cytoskeleton rearrangement in the cell membrane. In cold-treated tobacco cells
cellular or intercellular stress. In Arabidopsis thaliana, ADF genes occur in multiple gene families and are not conserved in animals and fungi. In maize, the ADF (ZmADF3) is phosphorylated by a calcium-stimulated protein kinase. ZmADF3 specifically binds to phosphoinositide (PIP2) to inhibit the enzymatic activity of phospholipase C (Gungabissoon et al. 1998, Smertenko et al. 1998). Post-translational modification of ADF by phosphorylation is essential for regulating its activity. Our results strongly suggest that ABA plays an important role in controlling the phosphorylation status of ADF protein, and thereby affects cytoskeletal rearrangement. It is not known whether the effect of ABA on ADF accounts, in part, for the effect of ABA on lateral root formation.

The role of ABA in regulating root growth and adaptation to environmental change

ABA plays a variety of roles in mediating plant root growth responses to environmental stresses, such as drought, chilling and high salinity, and nutritional stresses, such as phosphate deficiency and nitrate enrichment. In roots, an increase in endogenous ABA accompanies adaptive responses to environmental stresses. Exogenously applied ABA can help plants respond to, and survive environmental stress. However, the regulatory roles of ABA, and how it participates in adaptive processes, are not fully understood.

In Arabidopsis, ABA blocked lateral root formation just before activation of the lateral root meristem. In addition, a high concentration of NO3– inhibits lateral root formation and ABA participates in mediating the effect of NO3– (Signora et al. 2001). ABA also inhibits early lateral root development (Smet et al. 2003). Application of 10 μM ABA reduced lateral root formation 68% in Arabidopsis. However, the effects of ABA on rice seedling roots were very different. Instead of blocking lateral root formation, ABA promoted lateral root and root hair growth in rice seedlings. In cauliflower, ABA also increased its root hair density (Biddington and Dearman 1982). Two rice mutants (srt5 and srt6) in which lateral root elongation and root hair formation are extremely inhibited during the seedling stage have defects in either ABA biosynthesis or response (Yao et al. 2002, Yao et al. 2003). The srt5 mutant phenotype only appears during the seedling stage and the adult plant resembles the wild type. Seminal root growth of srt5 can be restored partially by exogenously applied ABA. In srt6, the response to ABA is deficient and lateral root elongation and root hair formation remain inhibited during all growth stages. Thus, endogenous ABA positively regulates early root growth in rice, and exogenously added ABA can partly compensate for deficiencies in endogenous ABA. The effect of ABA on lateral root formation in monocotyledons and dicotyledons remains a controversial issue.

Conclusion

A large amount of research has focused on the effect of auxin on lateral root formation in Arabidopsis. However, very little is known about the effect of ABA on root growth and development in rice. Our results indicate that ABA affects post-embryonic root growth and development in rice very differently from how it does in Arabidopsis. Our report is the first to demonstrate that exogenously applied ABA induced many aspects of changes in various root morphological features in rice seedling, including tip swelling, root hair formation and lateral root production. In addition, as indicated by the increased exudation volume and K+ content in xylem sap, ABA enhanced root cell vitality and increased water permeability. The response of roots to external ABA is highly dependent on an increase in cytoplasmic Ca2+, from either an extracellular or intracellular source. ABA also alters protein expression. It induces accumulation of ADF, which may end with the dynamic change of actin filaments and cause cytoskeleton rearrangements that are involved in the regulation of root tip growth. Based on these results, we developed a tentative model for how ABA affects the morphogenesis of rice root growth and development. First, ABA causes an increase in Ca2+ in the cytoplasm from both extracellular and intracellular sources. The Ca2+ ions then bind to calmodulins to form Ca2+-calmodulin complexes. The activated Ca2+-calmodulin complexes may activate other downstream signaling components, such as calcium/calmodulin-dependent protein kinase or phosphatase. This can lead to new protein synthesis, or change the phosphorylation status of ADF in root tips, to cause rearrangement of the cytoskeleton during cell growth. Finally, remodeling of the actin filaments may function as a stress mediator to regulate root tip swelling, root hair formation and, possibly, lateral root formation.

Materials and Methods

Plant materials

Rice (O. sativa L., cv. TN1) seeds were surface sterilized in 5% sodium hypochloride containing two drops of Tween-20 for 15 min followed by three washes in sterile water. Sterilized seeds were germinated in Petri dishes on moist filter paper at 37°C under dark conditions for 1 d. After germination, seeds were transferred to 600 ml beakers containing half-strength Kimura B solution and seedlings were grown in a growth chamber set for a daily regime of 30°C day (16 h) and 25°C night (8 h) for up to 10 d. Relative humidity was about 85%. For pharmacological tests, seedlings were placed on top of a centrifuge cap from which the center had been removed and replaced with steel mesh. Seeds were grown in 15 ml centrifuge tubes (four seedlings per tube) under the conditions described above. Care was taken to prevent the solution from drying up due to evaporation. For ABA treatment, rice seedling roots were treated hydroponically with 10 μM (±)-ABA.
Microscopy and sectioning

The typical paraffin method was used for anatomical analysis. Untreated and ABA-treated root tips were excised (1–1.5 cm segment) and fixed in FAA solution (formaldehyde : acetic acid : 70% alcohol, 5 : 5 : 90) for 24 h. Fixed tissues were washed with 50% ethanol for 30 min. Dehydration was performed gradually with increasing concentrations of t-butanol : 95% ethanol : H₂O (1 : 4 : 5), t-butanol : 95% ethanol : H₂O (2 : 5 : 3), t-butanol : 95% ethanol : H₂O (3.5 : 5 : 1.5), t-butanol : 95% ethanol (5.5 : 4.5) and t-butanol : absolute ethanol (7.5 : 2.5) for 2 h each, and t-butanol (100% + two drops of safranin) for 8–12 h.

For paraffin filtration, paraffin was added gradually to root materials at 60–65°C for 2 h. Another 8–12 h was taken to evaporate t-butanol. Roots were embedded in paraffin cakes, trimmed and cut into 8–10 μM sections with a rotary microtome.

Removal of paraffin and rehydration were begun by twice adding 100% xylene for 10 min, xylene : 100% ethanol (1 : 1) for 10 min, followed by a series of 100, 95, 85, 70 and 50% ethanol for 3 min each. Root sections were then stained with safranin for 18 h followed by two washes in 30% ethanol and sterile water, respectively. This safranin solution was replaced by Delafield’s hematoxylin for a second staining for 3–10 min before being washed twice in sterile water for 2 min. Roots were then dehydrated for 3 min each in 30, 50, 70, 85, 95 and 100% ethanol, followed by 3 min in absolute ethanol : xylene (1 : 1), and 5 min in 100% xylene, before being mounted in Euparal. All samples were photographed using an Olympus BX50 microscope equipped with a JVC KY-F558 camera. The images were processed using either Photoshop 5.0 or Microsoft Photo Editor.

TTC reduction assay

The TTC test was modified from the method of Steponkus and Lanphere (1967). Prior to TTC incubation, rice roots were washed with sterile water for 10 min and placed in 15 ml of TTC solution (0.08% TTC in 0.05 M sodium phosphate buffer, pH 7.4) for 24 h at 30°C in the dark. The TTC solution was drained and washed with sterile water. Roots for the measurement of TTC were excised and cut into 0.5% agarose in running buffer. SDS–PAGE was carried out using 2.5% polyacrylamide gel and overlaid with 0.5% agarose in running buffer. SDS–PAGE was carried out using 15% polyacrylamide gel and overlaid with 0.5% agarose in running buffer. SDS–PAGE was carried out using 20% polyacrylamide gel and overlaid with 0.5% agarose in running buffer. The gel was then subjected to in-gel reduction, alkylation and tryptic digestion (Tsai et al. 2000). Tryptic-digested peptides were recovered and then subjected to in-gel reduction, alkylation and tryptic digestion (Tsai et al. 2000). Tryptic peptide masses were measured with a gradient of 5% (v/v) to 65% (v/v) acetonitrile in 0.1% (v/v) formic acid over 60 min; chromatographed peptides were introduced at a flow rate of 5 μl/min into the electrospray source of a Finnigan LCQ ion trap mass spectrometer. The mass spectrometer was programmed to acquire successive sets of three scan modes and determined ion intensities from 395 to 1,605 m/z. The most abundant ions were analyzed by zoom scan and MS/MS scan. The resulting MS/MS spectra of the peptides were matched to the SWISS-PROT (http://kr.expasy.org/sprot/), NCBI (http://www.ncbi.nlm.nih.gov/), KOME (http://cdna01.dna.affrc.go.jp/CDNA/) and TIGR (http://tigrblast.tigr.org/tgi/) databases using the SEQUEST Blower software (Eng et al. 1994, Chittum et al. 1998).
Abscisic acid and root growth and development in rice

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References


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