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Research

Abscisic acid accumulation modulates auxin transport in the root tip to enhance proton secretion for maintaining root growth under moderate water stress

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Summary

- Maintenance of root growth is essential for plant adaptation to soil drying. Here, we tested the hypothesis that auxin transport is involved in mediating ABA's modulation by activating proton secretion in the root tip to maintain root growth under moderate water stress.
- Rice and Arabidopsis plants were raised under a hydroponic system and subjected to moderate water stress (−0.47 MPa) with polyethylene glycol (PEG). ABA accumulation, auxin transport and plasma membrane H^+-ATPase activity at the root tip were monitored in addition to the primary root elongation and root hair density.
- We found that moderate water stress increases ABA accumulation and auxin transport in the root apex. Additionally, ABA modulation is involved in the regulation of auxin transport in the root tip. The transported auxin activates the plasma membrane H^+-ATPase to release more protons along the root tip in its adaption to moderate water stress. The proton secretion in the root tip is essential in maintaining or promoting primary root elongation and root hair development under moderate water stress.
- These results suggest that ABA accumulation modulates auxin transport in the root tip, which enhances proton secretion for maintaining root growth under moderate water stress.

Introduction

Water stress is one of the major limiting factors for plant growth and development (Zhu, 2002; Chaves & Oliveira, 2004; Shane *et al.*, 2010). Plants must have evolved with some adaptive mechanisms to cope with water stress through certain biological processes (Davies & Zhang, 1991; Franks, 2011). Among these processes, root growth regulated by the sensory root tip plays an essential role in adaptive responses (Darwin, 1880; Baluska *et al.*, 2010; Chapman *et al.*, 2012). Under water stress, the root is the initial perceiver of water deficit in drying soil, followed by a series of responses at physiological, cellular and morphological levels (Sengupta *et al.*, 2011). Maintaining primary root elongation or root hair development has been shown to be an adaptive response to low water potentials (Sharp *et al.*, 1988; Schnall & Quatrano, 1992; Wu *et al.*, 1996; Yamaguchi & Sharp, 2010).

Root growth starts with cell wall extension at the cellular level. The acid growth theory implicates apoplastic protons (H^+) as the major wall-loosening factor causing cell extension (Rayle & Cleland, 1992; Fan & Neumann, 2004; Staal *et al.*, 2011). The production of apoplastic H^+ is mainly attributed to H^+ efflux mediated by the plasma membrane (PM) H^+-ATPase (Palmgren, 2001). The PM H^+-ATPase is mainly asymmetrically distributed in epidermal and outer cortical cells of plant roots (Jahn *et al.*, 1998; Haruta & Sussman, 2012). Optimal primary root growth or root hair development requires fine regulation of root tip H^+ secretion by the PM H^+-ATPase (Palmgren, 2001; Staal *et al.*, 2011; Haruta & Sussman, 2012). However, in roots, the components that fine-tune PM H^+-ATPase activity are unclear, especially under water stress.

Several plant hormones have been shown to regulate the adaptive responses of roots to fluctuating environments (Casson & Lindsey, 2003). Auxin plays a crucial role in root growth and development (Ribaut & Pilet, 1994; Fu & Harberd, 2003; Blilou *et al.*, 2005). Auxin transport is sufficient to generate the auxin maxima and gradients that can guide root growth (Grieneisen *et al.*, 2007; Robert & Friml, 2009). Additionally, auxin plays a role in H^+ secretion by regulating the activity of the PM H^+-ATPase (Rober-Kleber *et al.*, 2003; Staal *et al.*, 2011). Just like auxin, the stress-induced ABA also plays an important role in regulating root growth (Sharp, 2002; Zhang *et al.*, 2010). ABA accumulates rapidly in roots under water stress (Sengupta *et al.*, 2011). Increased accumulation of ABA towards the root tip is required for maintaining primary root elongation at low water

potentials (Saab *et al.*, 1990; Sharp *et al.*, 1994). Furthermore, maintenance of root elongation by ABA is conferred by its regulatory functions in ion homeostasis, osmotic adjustment and cell wall extensibility (Yamaguchi & Sharp, 2010). The question remains as to how auxin's and ABA's actions are integrated into root growth and development under water stress.

Interactions between auxin- and ABA-dependent responses have been described in roots. For example, in Arabidosis plants, the *ABI3* (abscisic acid insensitive 3) gene is involved in auxin signalling and root development (Brady *et al.*, 2003). In addition, there may be crosstalk between ABA and auxin signalling pathways to control root development (Rock & Sun, 2005). ABA might play a role in modulating auxin distribution in the elongation zone of the root tip under water stress (Yamaguchi & Sharp, 2010). In this study, we hypothesize that auxin transport plays an important role in mediating the regulation of ABA on root tip adaption by modulating proton secretion under water stress. Rice (monocotyledonous model plant) and Arabidopsis (dicotyledonous model plant) were used in a hydroponic system. Our results suggest that ABA accumulation modulates auxin transport in the root tip, which enhances proton secretion to maintain primary root elongation and root hair development under water stress.

Materials and Methods

Plant materials, growth conditions, and stress treatment

The wildtype (WT) Arabidopsis (*Arabidopsis thaliana* (L.) Heynh) was of ecotype Col-0 unless otherwise indicated. Some of the Arabidopsis plant material used in this study was described in our previous papers: *aba3-1* (Jia *et al.*, 2012), *aux1-7* (Li *et al.*, 2010) and the AUX1:YFP line (Li *et al.*, 2011). The *pin2* mutant (*eir1-4*), PIN2:GFP line and DR5rev:GFP line were kindly provided by Jiri Friml (Department of Plant Systems Biology, Flanders Institute for Biotechnology, Belgium). Some stocks of Arabidopsis plants (*aha2* mutant: Salk_082786; natural accessions: Lov-5, Uod-1, Uod-7 and Ws-2) were obtained from the Arabidopsis Biological Resource Center (ABRC, Ohio State University, Columbus, OH, USA). The homozygous *aha2* mutant was identified by PCR using primers specific to the T-DNA of Salk_082786 (Supporting Information, Table S1).

Arabidopsis plants were grown hydroponically as described by Xu & Shi (2008). Rice plants (*Oryza sativa* L. Japonica nipponbare) were grown hydroponically according to the method of Xu *et al.* (2010). Since polyethylene glycol 8000 (PEG8000, Sigma, St Louis, MO, USA) can induce oxygen deficiency and inhibit root growth in hydroponic systems (Verslues *et al.*, 1998), we adopted the multi-points and multidirectional aeration system (four aeration equipments were fixed along the four walls of the hydroponic pot) to increase oxygen movement or oxygen availability in this hydroponic system. The seminal root of 15-d-old rice seedlings was considered as the primary root (Yao *et al.*, 2003). Rice or Arabidopsis plants (15-d-old) were transferred to the nutrient solution with or without the following additions: PEG 8000, ABA, the ABA biosynthetic inhibitor fluridone (FLU), the auxin influx inhibitor 3-chloro-4-hydroxyphenylacetic acid (CHPAA), the auxin efflux inhibitor 1-naphtuylphthalamic acid (NPA), and the PM ATPase inhibitor vanadate (VAN) treatments for 12, 24, 36, 48 and 96 h.

Analysis of root elongation and root hair density

Primary root length was measured using a root analysis instrument (WinRHIZO; Regent Instruments Inc., Quebec, ON, Canada) according to the method of Xu & Shi (2007). The primary root elongation rate of rice (mm h^{-1}) or Arabidopsis (μm h^{-1}) was calculated from the primary root length with respect to the displacement of primary root apex for the duration of control or experimental treatment. Root hairs were determined in the region 0–5000 μm from the root cap junction in rice or Arabidopsis plants using confocal laser scanning microscopy (Olympus FV-1000 spectral type SPD mar/G/R IX81 FLUOVIEW laser confocal system) according to the method of Santi &

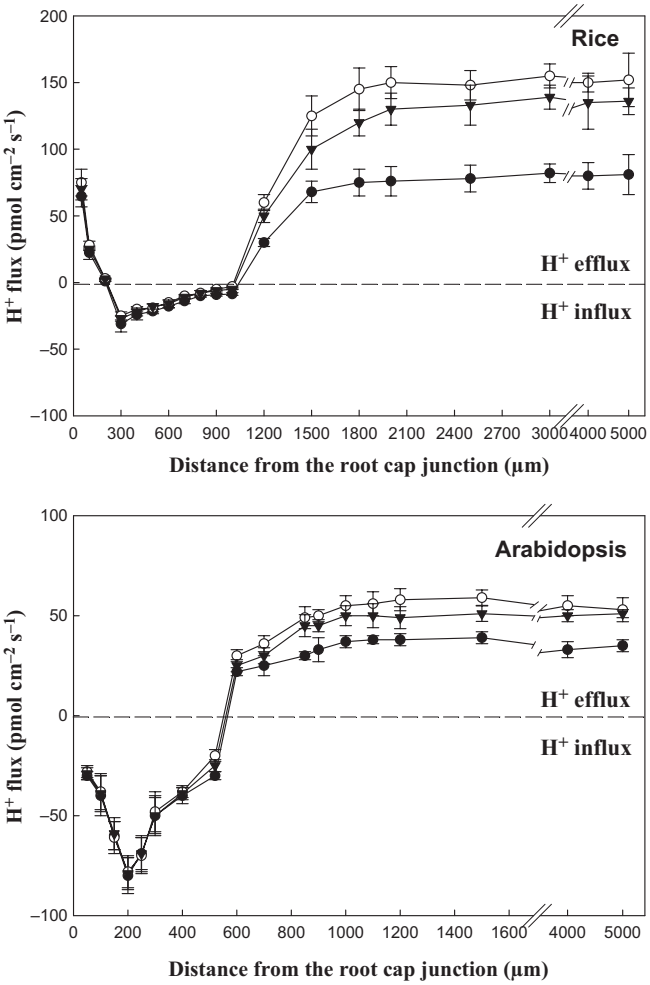

Fig. 1 Effects of moderate water stress or exogenous ABA treatment on H^+ flux along the root tip of rice or Arabidopsis plants (0–5000 μm from the root cap junction). Fifteen-day-old rice or Arabidopsis plants were exposed to control conditions (●, control), moderate water stress (○, 5% PEG 8000) or exogenous ABA (▼, 0.1 μM) for 24 h in hydroponics. The values are the means and SD of six replicates from two independent experiments.

Schmidt (2009). Root hair densities were estimated from a $100 \times 100\ \mu m^2$ section along the region 0–5000 μm as averages.

Assay of plasma membrane H^+-ATPase activity or H^+ flux along root tip

Plasma membrane H^+-ATPase activity of the root tip (0–5000 μm from the root cap junction) was determined according to the method of Xu *et al.* (2012). H^+ fluxes were measured noninvasively using the scanning ion-selective electrode technique (SIET; SIET system BIO-003A; Younger USA Science and Technology Corporation; Applicable Electronics Inc.; Science Wares Inc., Falmouth, MA, USA) according to the method of Xu *et al.* (2012). The H^+ fluxes of rice plants were measured along the root tip, concentrating on the following zones: 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1200, 1500, 1800, 2000, 2500, 3000, 3500, 4000 and 5000 μm from the root cap junction. The H^+ fluxes of Arabidopsis plants were measured along the root tip, concentrating on the following zones: 50, 100, 150, 200, 250, 300, 400, 520, 600, 700, 850, 900, 1000, 1100, 1200, 1500, 2000, 3000, 4000 and 5000 μm from the root cap junction.

Determination of ABA concentration

Determination of endogenous ABA concentrations of root tips (0–5000 μm from root cap junction) was carried out using the radioimmunoassay method as described by Ye *et al.* (2012). Root samples (0.2 g) were homogenized in 1 ml of distilled water and then shaken at 4°C overnight. The homogenates were centrifuged at 12 000 ***g*** for 10 min at 4°C and the supernatant was used directly for the ABA assay. The 450 μl reaction mixture contained 200 μl of phosphate buffer (pH 6.0), 100 μl of diluted antibody (Mac 252) solution, 100 μl of [^{3}H] ABA (8000 cpm) solution, and 50 μl of crude extract. The mixture was then incubated at 4°C for 45 min and the bound radioactivity was measured in 50% saturated $(NH_4)_2SO_4$-precipitated pellets with a liquid scintillation counter.

Assay of auxin content and auxin transport

Auxin (IAA) content was analyzed by GS-selected reaction monitoring mass spectrometry as described by Ljung *et al.* (2005). The root tips (0–5000 μm from the root cap junction) were collected, and then six replicates of the samples were purified after addition of 250 pg of $^{13}C_6$-IAA internal standard. For the analysis of root basipetal auxin transport in rice or Arabidopsis plants, 1% (w/v) agar blocks containing 100 nM ^{3}H-IAA were placed in contact with the root tips (0–5000 μm from the root cap junction) for 5 h in the dark. The apical 2 mm of the roots was discarded, and the apical 3 mm sections of the remaining roots were excised for radioactivity counting, as described by Li *et al.* (2011).

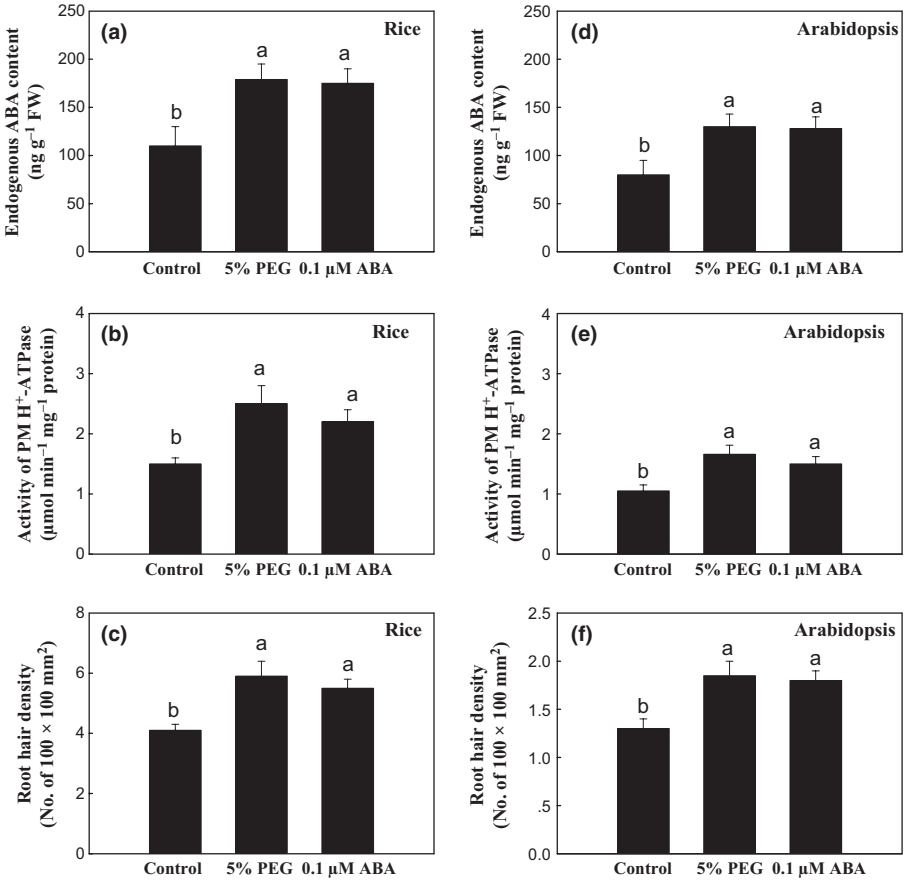

Fig. 2 Effects of moderate water stress or exogenous ABA treatment on the endogenous ABA concentration, activity of plasma membrane (PM) H^+-ATPase and root hair density in the root tip of rice (a–c) or Arabidopsis plants (d–f). Fifteen-day-old rice or Arabidopsis plants were exposed to control conditions (control), moderate water stress (5% polyethylene glycol 8000 (PEG 8000) or treated with exogenous ABA (0.1 μM) for 24 h in hydroponics. Measurements were taken at 0–5000 μm from the root cap junction. The values are the means and SDs of six replicates from two independent experiments. A ranking test was performed for a, b or c (lowercase letter indicated above each column) in figures, and different letters indicate significant differences at the $P < 0.05$ level.

Confocal laser microscopy

The fluorescence of DR5rev:GFP, AUX1:YFP or PIN2:GFP in Arabidopsis root tip was observed (with the same confocal microscope as described earlier) according to the method of Sun *et al.* (2011). To image green and yellow fluorescent protein (GFP and YFP), the 488 and 514 nm lines of the argon laser were used for excitation, and emission was detected at 510 and 530 nm, respectively. Approx. 10 seedlings/image were examined, and at least two independent experiments were performed, giving the same statistically significant results. All images were taken under the same conditions.

Reverse transcription polymerase chain reaction (RT-PCR)

Reverse transcription polymerase chain reaction was assayed according to the method of Xu & Shi (2006). Total RNA was extracted from Arabidopsis plants. Gene sequences were available in at the National Center of Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov) and gene-specific primers for real-time RT-PCR were designed using Primer 5 software (Table S1). *At-ACT2* is a strongly and constitutively expressed 'housekeeping' gene in Arabidopsis plants (Xu *et al.*, 2012), so the quantification of mRNA was based on comparison with the level of mRNA for *At-ACT2*.

Statistical analyses

Data were subjected to ANOVA, and *post hoc* comparisons were done with Duncan's multiple range test at the $P < 0.05$ level. The statistical software program used was SPSS, version 13.0 (SPSS Inc., Chicago, IL, USA). The values are the means and SD of six replicates from two independent experiments. A ranking test was performed for a, b or c (lowercase) in figures and different letters indicate significant differences at the $P < 0.05$ level.

Results

Root tip response to moderate water stress or exogenous ABA treatment

Primary root elongation rates in rice or wildtype Arabidopsis were first analysed under water stress (PEG 8000 at 0 and 5% at −0.47 MPa, 7.5% at −0.91 MPa, 10% at −1.48 MPa, 15% at −3.02 MPa and 20% at −5.11 MPa) for 12, 24, 36, 48 and 96 h (Fig. S1). The results show that, compared with 0% PEG 8000, 5% significantly increased the primary root elongation rates over the entire treatment ($P < 0.05$). According to van der Weele *et al.* (2000), water potentials ranging from −0.23 to −0.51 MPa impose 'moderate water stress' in Arabidopsis

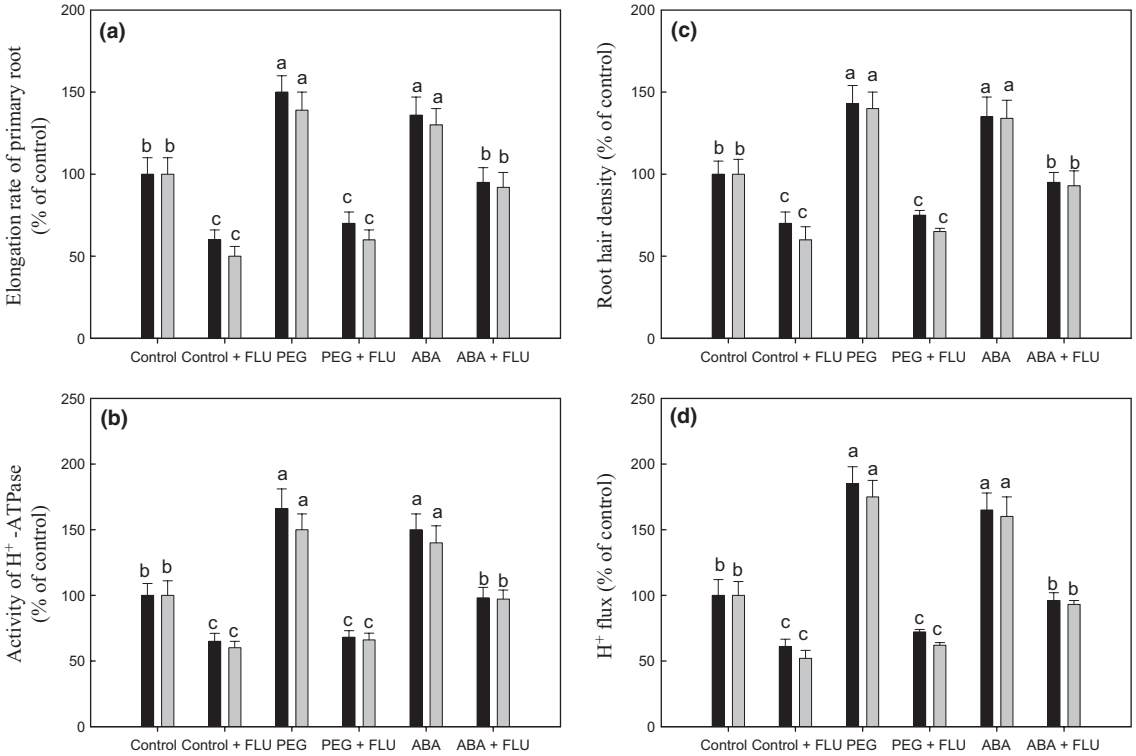

Fig. 3 Effect of fluridone (FLU, an ABA biosynthetic inhibitor) on the root tip response of rice (black bars) or Arabidopsis (grey bars) to moderate water stress or exogenous ABA treatment. Fifteen-day-old rice or Arabidopsis plants were exposed to control conditions (control), moderate water stress (5% PEG 8000) or treated with exogenous ABA (0.1 μM) containing FLU (fluridone: 10 μM) for 24 h in hydroponics. (a) Primary root elongation rate; (b) plasma membrane (PM) H^+-ATPase activity in the root tip (0–5000 μm from the root cap junction); (c) root hair density in the root tip (0–5000 μm from the root cap junction); (d) H^+ efflux at 1500 μm from the root cap junction. Values from rice or Arabidopsis plants under control conditions are expressed as 100%, and their absolute control data are shown in Table S2. The values are the means and SDs of six replicates from two independent experiments. A ranking test was performed for a, b or c in figures, and different letters (above bars) indicate significant difference at the $P < 0.05$ level.

plants, and primary root elongation during the first 3–4 d of this 'moderate water stress' is significantly greater than that in normal growth conditions. Here, the water potential set by 5% PEG 8000 is −0.47 MPa (Michel, 1983) and equivalent to 'moderate water stress'. Thus, our results in rice or Arabidopsis were in agreement with the results of van der Weele *et al.* (2000).

We also compared the effects with that of ABAs. Primary root elongation in rice or Arabidopsis plants was studied with different concentrations of ABA (0, 0.1, 0.2, 1, 5 and 10 μM ABA) for 24 h (Fig. S2). The results show that, compared with 0 μM ABA, treatment with exogenous 0.1 μM ABA significantly increased the primary root elongation rates in rice or Arabidopsis plants ($P < 0.05$). Therefore, we selected 5% PEG 8000 (moderate water stress) and exogenous 0.1 μM ABA for 24 h treatments in our later experiments.

Under the control condition (control), moderate water stress (5% PEG 8000) or mild exogenous ABA treatment (0.1 μM ABA), H^+ fluxes were measured along the root tip (0–5000 μm from the root cap junction) of rice or wildtype Arabidopsis (Fig. 1). Compared with the control, PEG or ABA treatment for 24 h significantly increased H^+ efflux at 1200–5000 μm distance from the root cap junction in rice and increased H^+ efflux at 850–5000 μm distance from the root cap junction in Arabidopsis. Thus, we selected 1500 μm from the root cap junction to analyse H^+ secretion at the root tip of rice or Arabidopsis for further experiments. According to Fig. 2, compared with the control, PEG and ABA treatment for 24 h also significantly increased the endogenous ABA content, PM H^+-ATPase activity and root hair density in the root tip (0–5000 μm from the root cap junction) in both rice and Arabidopsis.

ABA modulation in root tip response to moderate water stress

Fluridone, an ABA biosynthetic inhibitor (10 μM; Yoshioka *et al.*, 1998), was used to investigate the effect of ABA on root tip responses under moderate water stress in rice and Arabidopsis. The PEG or ABA treatment for 24 h significantly increased the primary root elongation rate, PM H^+-ATPase activity, root hair density, and H^+ efflux, while FLU treatment significantly suppressed these responses (Fig. 3). We also tested the ABA biosynthetic mutant of Arabidopsis, *aba3-1*, to examine the effect of ABA on root tip responses under moderate water stress (Fig. 4). Under control, PEG and ABA treatments, the primary root elongation rate, PM H^+-ATPase activity, root hair density and proton efflux were significantly lower in the *aba3-1* mutant than in wildtype Arabidopsis.

Auxin transport in the root tip in response to moderate water stress

In addition to ABA, auxin is also an important hormone in responding to water stress in plant roots (Ribaut & Pilet, 1994; Seo *et al.*, 2009). Thus, in this study, root tip responses of rice and Arabidopsis were investigated under control, PEG and ABA treatments containing CHPAA (auxin influx inhibitor; 10 μM)

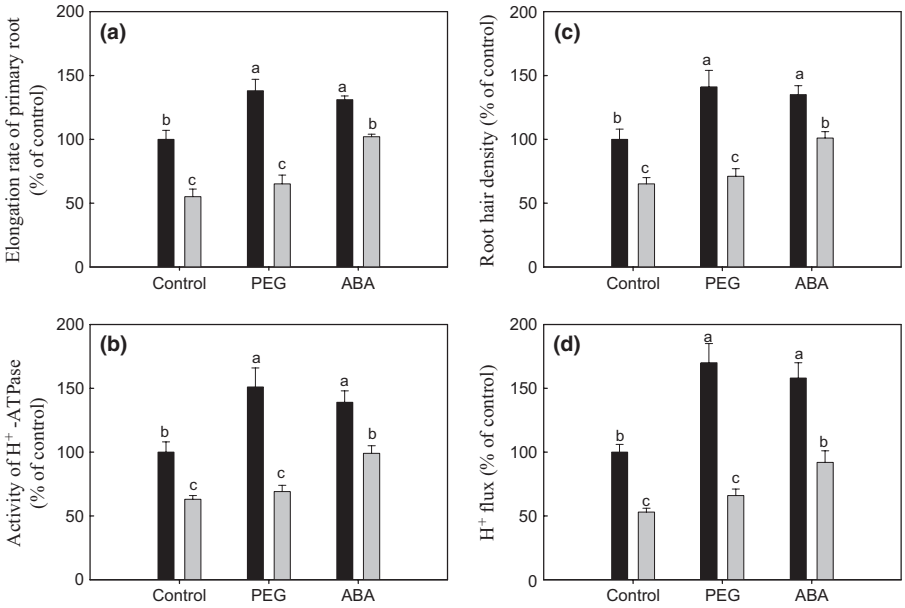

Fig. 4 Root tip response of wildtype Arabidopsis (black bars) or *aba3-1* (grey bars) mutant to moderate water stress or exogenous ABA treatment. Fifteen-day-old Arabidopsis plants were grown under control conditions (control), moderate water stress (5% polyethylene glycol 8000 (PEG 8000)) or with exogenous ABA (0.1 μM) for 24 h in hydroponics. (a) Primary root elongation rate; (b) plasma membrane (PM) H^+-ATPase activity in the root tip (0–5000 μm from the root cap junction); (c) root hair density in the root tip (0–5000 μm from the root cap junction); (d) H^+ efflux at 1500 μm from the root cap junction. Values from wildtype plants under control conditions (control) are expressed as 100%, and these absolute control data are shown in Table S2. The values are the means and SDs of six replicates from two independent experiments. A ranking test was performed for a, b or c in figures, and different letters (above bars) indicate significant difference at the $P < 0.05$ level.

or NPA (auxin efflux inhibitor; 10 μM; Lankova *et al.*, 2010). As shown in Fig. 5, CHPAA and NPA treatment significantly reduced the root elongation rate, root hair density, PM H^+-ATPase activity and H^+ efflux under all treatments. Additionally, compared with the control, both PEG and ABA treatment significantly increased the transcript abundance of *AUX1* (an auxin influx transporter) and *PIN2* (an auxin efflux transporter) in the root tip of Arabidopsis (Fig. S3). Also, both PEG and ABA treatment increased the abundance of AUX1:YFP and PIN2:GFP in the Arabidopsis root tip compared with the control (Fig. S4). Moreover, using *aux1-7* (*aux1* Arabidopsis mutant) and *eir1-4* (*pin2* Arabidopsis mutant), we found that, compared with wildtype Arabidopsis plants, the root elongation rate, root hair density, PM H^+-ATPase activity and proton efflux were significantly lower in *aux1-7* or *eir1-4* mutant plants under control, PEG or ABA treatments (Fig. 6).

In addition, using *aha2* (PM H^+-ATPase isoform 2-deficient Arabidopsis mutant), we found that although the root elongation rate, root hair density and proton efflux were significantly lower in the *aha2* mutant than in wildtype Arabidopsis under control, PEG and ABA treatments, there were no significant differences in these parameters between the wildtype and *aha2* under control, PEG and ABA treatments containing CHPAA or NPA (Fig. 7a,b,d). However, in wildtype Arabidopsis and the *aha2*

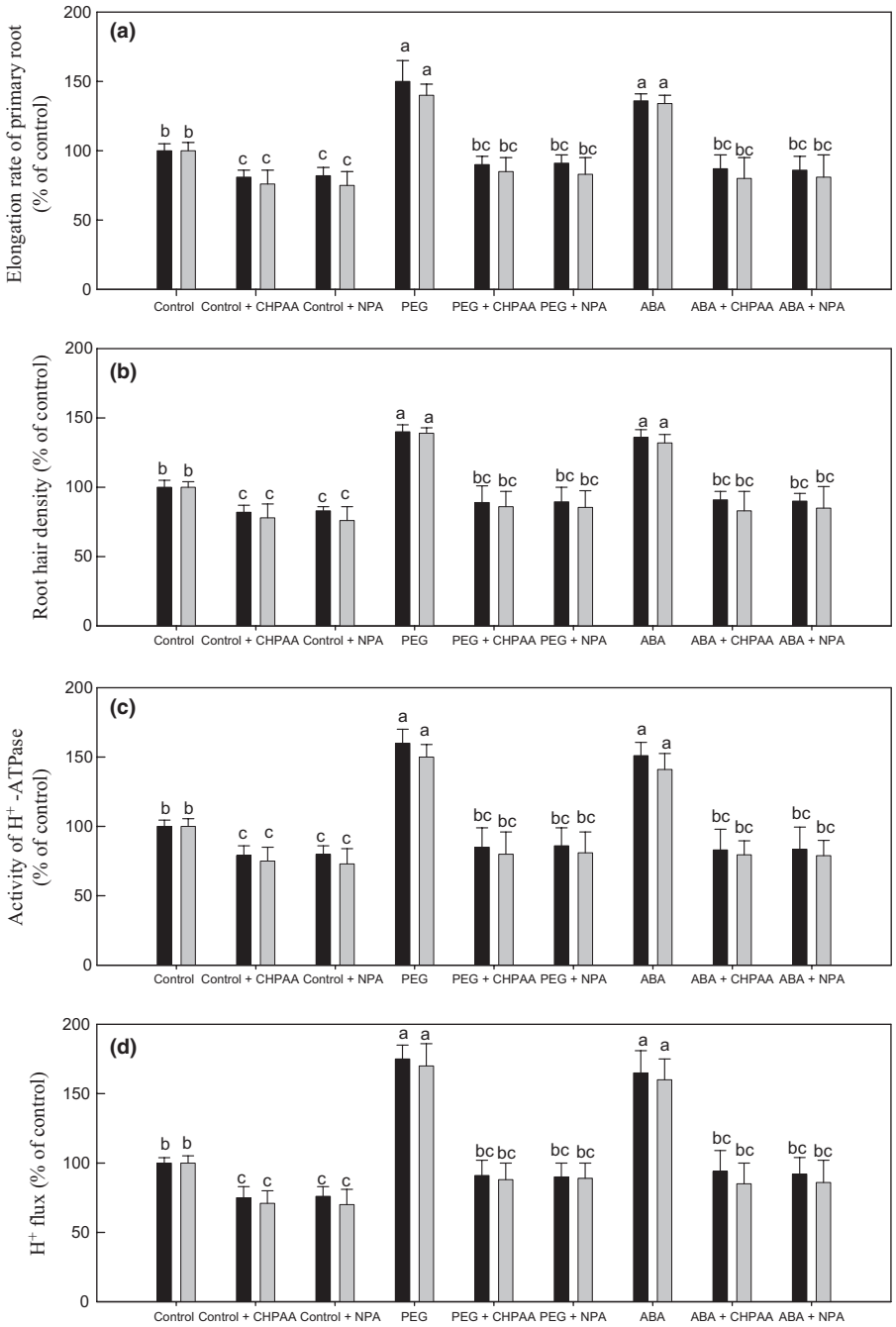

Fig. 5 Root tip response of rice (black bars) or Arabidopsis (grey bars) plants under moderate water stress or exogenous ABA treatment. Fifteen-day-old rice or Arabidopsis plants were treated under control conditions (control), moderate water stress (5% polyethylene glycol 8000 (PEG 8000)) or with exogenous ABA (0.1 μM) containing CHPAA (an auxin influx inhibitor; 10 μM) or NPA (an auxin efflux inhibitor; 10 μM) for 24 h in hydroponics. (a) Primary root elongation rate; (b) root hair density in the root tip (0–5000 μm from the root cap junction); (c) plasma membrane (PM) H^+-ATPase activity in the root tip (0–5000 μm from the root cap junction); (d) H^+ efflux at 1500 μm from the root cap junction. Elongation rate, root hair density, PM H^+-ATPase activity and H^+ efflux in rice or Arabidopsis plants under control conditions are plotted as 100%. The absolute control data are shown in Table S2. The values are the means and SDs of six replicates from two independent experiments. A ranking test was performed for a, b or c in figures, and different letters (above bars) indicate significant difference at the $P < 0.05$ level.

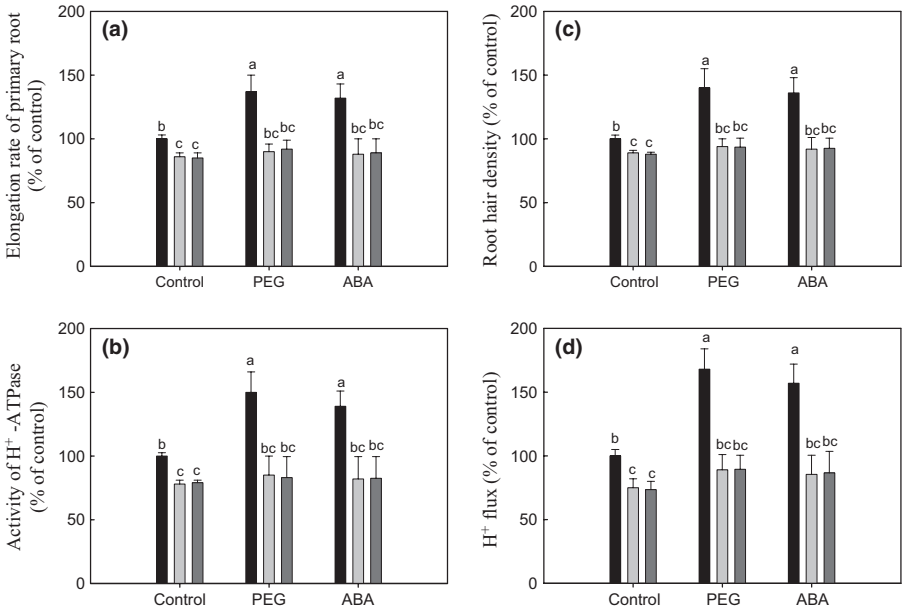

Fig. 6 Root tip response of wildtype Arabidopsis plants (black bars), *aux1-7* (light grey bars) or *eir1-4* mutant Arabidopsis plants (dark grey bars) under moderate water stress or exogenous ABA treatment. Fifteen-day-old Arabidopsis plants were treated under control conditions (control), moderate water stress (5% polyethylene glycol 8000 (PEG 8000)) or with exogenous ABA (0.1 μM) for 24 h in hydroponics. (a) Primary root elongation rate; (b) plasma membrane (PM) H^+-ATPase activity in the root tip (0–5000 μm from the root cap junction); (c) root hair density in the root tip (0–5000 μm from the root cap junction); (d) H^+ efflux at 1500 μm from the root cap junction. Elongation rate, root hair density, PM H^+-ATPase activity and H^+ efflux in wildtype Arabidopsis plants under control conditions are plotted as 100%, and these absolute control data are shown in Table S2. The values are the means and SDs of six replicates from two independent experiments. A ranking test was performed for a, b or c in figures, and different letters (above bars) indicate significant difference at the $P < 0.05$ level.

mutant, addition of CHPAA or NPA did not significantly affect the endogenous ABA content under these treatments (Fig. 7c).

In further experiments, we first used the auxin-responsive report DR5rev:GFP to study the abundance and distribution of auxin in the root tip of wildtype Arabidopsis (DR5rev:GFP). Although there was no significant difference in auxin abundance in the root tip of wildtype Arabidopsis under control, PEG or ABA treatment, the PEG and ABA treatments significantly increased the auxin distribution in the epidermal and cortical cells of the root tip compared with the control (Fig. S4, as shown by the white arrowheads). Based on the radioactive assay of IAA, our results also show that in rice or Arabidopsis, there was no significant difference in auxin content of the root tips under control, PEG or ABA treatment, while the PEG and ABA treatments significantly increased the basipetal auxin transport in root tips compared with the control (Fig. 8).

Root tip response of different natural accessions of Arabidopsis to moderate water stress

Santi & Schmidt (2009) reported that two natural Arabidopsis accessions (Uod-1 and Ws-2) have high rhizosphere acidification capacity, while two other accessions (Uod-7 and Lov-5) have low rhizosphere acidification capacity. According to Fig. S5, under moderate water stress (5% PEG for 24 h), the H^+ efflux, endogenous ABA content, primary root elongation rate and root hair density in Uod-1 and Ws-2 were significantly higher than in Uod-7 and Lov-5 (Fig. S5A,C,D,F). Also, compared with Uod-7 or Lov-5, the transcript abundance of *AUX1* and *PIN2* in Uod-1 or Ws-2 was significantly higher under moderate water stress (Fig. S5B,E). Thus, among these natural Arabidopsis accessions, we selected Uod-1 (Ottenhof, Austria) and Uod-7 (Ottenhof, Austria) for further experimentation.

As shown in Fig. 9, under PEG treatment, the endogenous ABA content, root basipetal auxin transport and proton efflux in Uod-1 were significantly higher than in Uod-7. Further, under PEG + FLU treatment, there were no significant differences in the three parameters between Uod-1 and Uod-7. Under PEG + CHPAA or NPA treatment, although the endogenous ABA content in Uod-1 was significantly higher than in Uod-7, no significant difference was found for root basipetal auxin transport or proton efflux. Further, under PEG + VAN treatment, the endogenous ABA content and root basipetal auxin transport in Uod-1 were significantly higher than in Uod-7, while there was no significant difference in proton efflux between Uod-1 and Uod-7.

Discussion

Proton secretion is involved in the adaption of the root tip to moderate water stress

The growth of roots in response to water stress is more adaptive (less inhibited) than that of shoots. Under mild water stress, shoot growth is inhibited, while primary root elongation is maintained or even promoted (van der Weele *et al.*, 2000; Sharp &

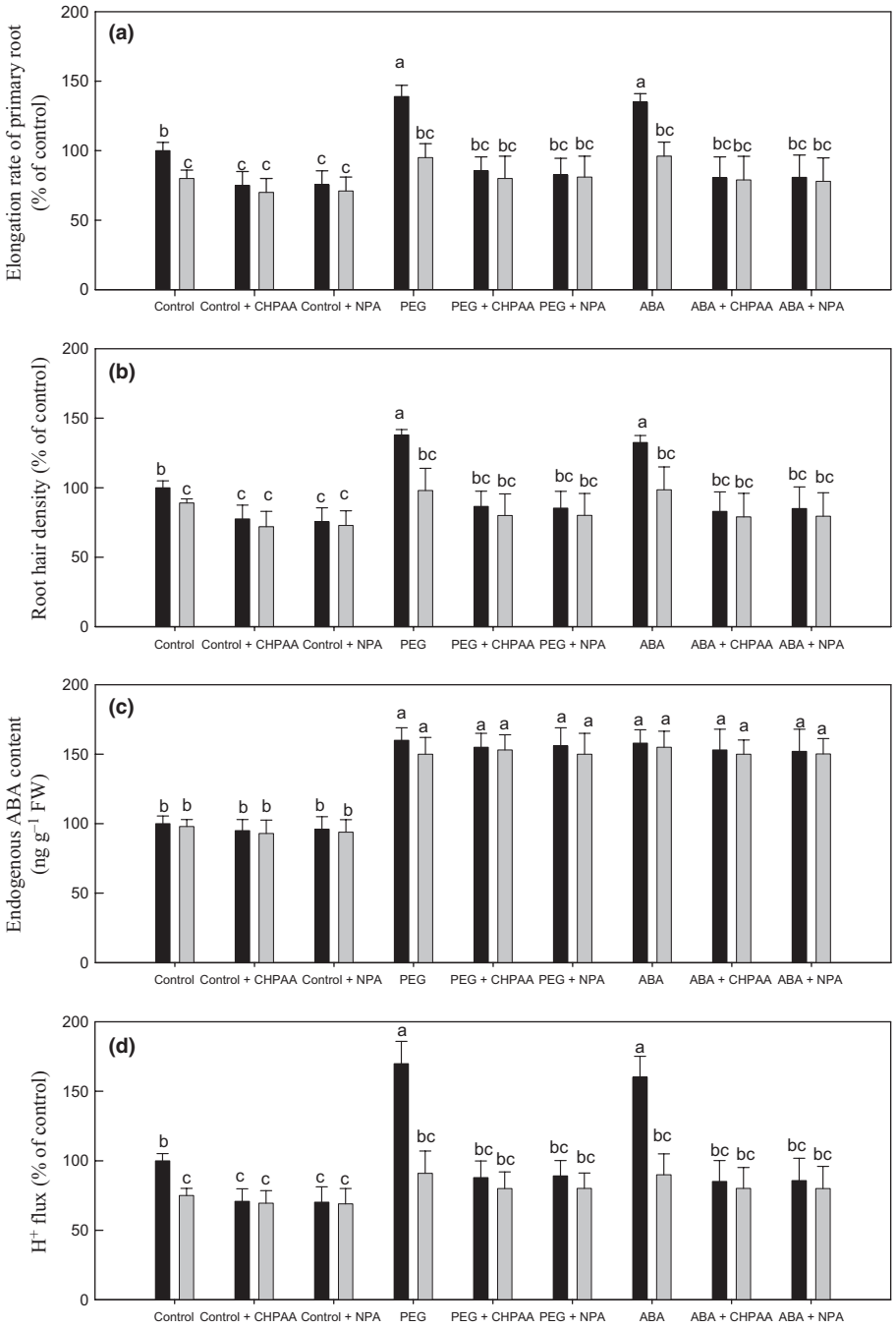

Fig. 7 Root tip response of wildtype (black bars) Arabidopsis plants or *aha2* mutant Arabidopsis plants (grey bars) to moderate water stress or exogenous ABA treatment. Fifteen-day-old Arabidopsis plants were treated under control conditions (control), moderate water stress (5% polyethylene glycol 8000 (PEG 8000)) or with exogenous ABA (0.1 μM) containing CHPAA (an auxin influx inhibitor; 10 μM) or NPA (an auxin efflux inhibitor; 10 μM) for 24 h in hydroponics. (a) Primary root elongation rate; (b) root hair density in the root tip (0–5000 μm from the root cap junction); (c) endogenous ABA content in the root tip (0–5000 μm from the root cap junction); (d) H^+ efflux at 1500 μm from the root cap junction. Elongation rate, root hair density, ABA content and H^+ efflux in wildtype Arabidopsis plants under control conditions are plotted as 100%, and the absolute control data are shown in Table S2. The values are the means and SDs of six replicates from two independent experiments. A ranking test was performed for a, b or c in figures, and different letters (above bars) indicate significant difference at the $P < 0.05$ level.

LeNoble, 2002). Root hair development is also a sensitive phenotype in response to water stress (Schnall & Quatrano, 1992). It has been suggested that proton secretion mediated by PM H^+-ATPases plays a key role in primary root elongation or root hair development (Santi & Schmidt, 2009). Thus, we hypothesized that root proton secretion is involved in the response to water stress. We found that compared with control conditions, moderate water stress promoted the root tip in adaptation to water stress in rice or Arabidopsis (Figs 1, 2, S1, S2). Under moderate water stress, when PM H^+-ATPase was inhibited (in rice, Fig. S6) or deficient (in Arabidopsis, Figs 7, 9), the proton efflux in root tips was greatly reduced, and primary root elongation or root hair density was also significantly decreased in root tip. Thus, these results suggest that proton secretion is regulated in the adaptation to moderate water stress and maintains or promotes primary root elongation and root hair development.

ABA modulation and auxin transport regulate the proton secretion in root tip

Although some research suggests that ABA plays an important role in maintaining or promoting root growth and development under water stress (Spollen *et al.*, 2000; Sharp, 2002; Sharp *et al.*, 2004; Yamaguchi & Sharp, 2010; Zhang *et al.*, 2010), the

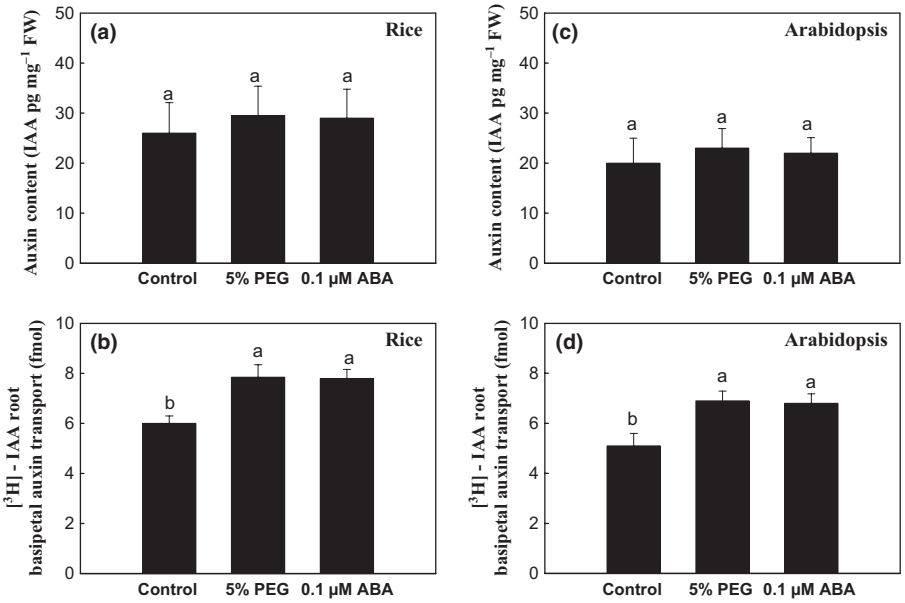

Fig. 8 Effects of moderate water stress or exogenous ABA treatment on the endogenous auxin (IAA) concentration and root basipetal auxin transport in the root tip of rice plants (a, b) or Arabidopsis plants (c, d). Fifteen-day-old rice or Arabidopsis plants were treated under control conditions (control), moderate water stress (5% polyethylene glycol 8000 (PEG 8000)) or with exogenous ABA (0.1 μM) for 24 h in hydroponics. (a, c) Endogenous auxin (IAA) content in the root tip (0–5000 μm from the root cap junction); (b, d) basipetal auxin transport in the root tip was measured using ^{3}H-IAA-labelling assays. The values are the means and SDs of six replicates from two independent experiments. A ranking test was performed for a, b or c in figures, and different letters (above bars) indicate significant difference at the $P < 0.05$ level.

detailed adaptive mechanism is still not well elucidated. We found that just like moderate water stress, exogenous ABA treatment also increased proton secretion in root tips (Figs 1, 2, S2). Under moderate water stress or exogenous ABA treatment, when ABA biosynthesis was inhibited or deficient in either rice or Arabidopsis (Figs 3, 4), proton secretion in root tips was greatly decreased, and primary root elongation, root hair density and PM H^+-ATPase activity were also significantly reduced. These results suggest that ABA modulation plays an important role in root tip adaption to moderate water stress by activating proton secretion.

Auxin gradient and accumulation mediated by auxin transport play important roles in the regulation of root growth and development and may also regulate proton secretion and the PM H^+-ATPases (Pitts *et al.*, 1998; Blilou *et al.*, 2005; Jones *et al.*, 2009; Staal *et al.*, 2011). We found that when auxin influx or efflux transport was inhibited or deficient in rice or Arabidopsis (Figs 5, 6), proton secretion in root tips was greatly decreased under control or moderate water stress or exogenous ABA treatment. Accordingly, primary root elongation, root hair density and PM H^+-ATPase activity were also significantly decreased in the root tip. All these results suggest that auxin transport mediates root tip adaptation to moderate water stress with the enhancement of proton secretion.

Involvement of auxin transport in the integration of ABA modulation

These results suggest that proton secretion changes as an adaptation of the root tip to moderate water stress. ABA modulation and auxin transport play an important role in this process. What is the relationship between ABA modulation, auxin transport and proton secretion in the adaptation of root tips to moderate water stress? First, PM H^+-ATPase-mediated proton secretion has been proved to be the downstream target of auxin signalling in promoting cell elongation (Rober-Kleber *et al.*, 2003). We found that under water stress, when auxin transport was inhibited or deficient, the activities of PM H^+-ATPase and proton efflux were substantially reduced (Figs 5, 6), and that auxin transport affects PM H^+-ATPase-mediated proton secretion under water stress (Fig. 7). Secondly, MYB96-mediated ABA signalling (MYB96: drought-induced transcription factor) is transduced through an auxin signal pathway during drought response in Arabidopsis (Seo *et al.*, 2009). We found that when auxin transport was inhibited or deficient in rice or Arabidopsis, exogenous ABA treatment or water stress could not improve PM H^+-ATPase-mediated proton secretion (Figs 5, 6).

Some researchers have suggested that the interaction between ABA and auxin plays an important role in plant growth and development (Belin *et al.*, 2009; Yang *et al.*, 2011). In this study, we found that ABA signalling results in enhanced auxin transport under water stress (Figs 8, S3, S4). Further, under moderate water stress, using Arabidopsis natural accessions (Fig. 9), we found that when ABA biosynthesis was inhibited, the endogenous ABA content, root basipetal auxin transport and proton efflux in Uod-1 (high root-tip adaptation capacity) were no longer significantly higher than those in Uod-7 (low root-tip adaption capacity). When auxin transport was inhibited, the endogenous ABA content in Uod-1 was still higher than in Uod-7. Root basipetal auxin transport and proton efflux in Uod-1 were no longer significantly higher than those in Uod-7. When PM H^+-ATPase was inhibited, proton secretion in Uod-1 was no longer significantly higher than in Uod-7. The endogenous ABA content and root basipetal auxin transport in Uod-1 were still higher than in Uod-7. Meanwhile, the results of Fig. S6 (in rice) were in agreement with those responses in Fig. 9. Taken together, all these results suggest that ABA induces auxin transport to regulate proton secretion in the root tip under moderate water stress.

Additionally, some previous studies have suggested that ABA accumulation restricts ethylene synthesis in water-stressed roots, and that ethylene can also interact with auxin synthesis and

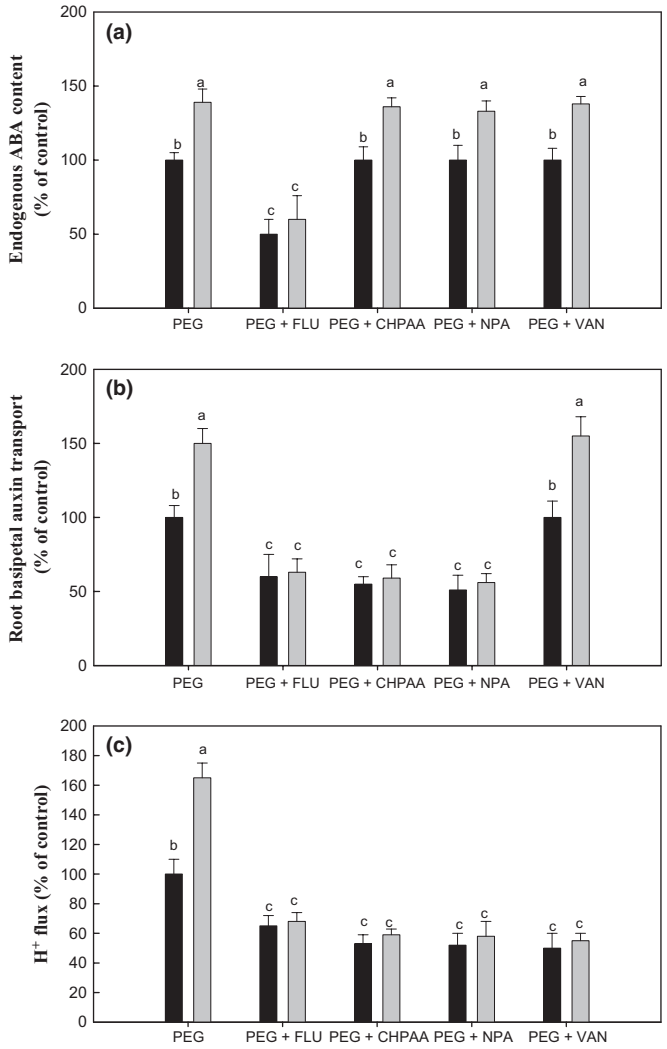

Fig. 9 Root tip response of Uod-7 (black bars) or Uod-1 (grey bars) Arabidopsis plants to moderate water stress. Fifteen-day-old Arabidopsis plants were exposed to moderate water stress (5% polyethylene glycol 8000 (PEG 8000)) and moderate water stress combined with fluridone (FLU, an ABA biosynthetic inhibitor; 10 μM), CHPAA (an auxin influx inhibitor; 10 μM), NPA (an auxin efflux inhibitor; 10 μM) or VAN (a plasma membrane ATPase inhibitor; 1 mM vanadate) for 24 h in hydroponics. (a) Endogenous ABA content in the root tip (0–5000 μm from the root cap junction); (b) basipetal auxin transport in the root tip; (c) H^+ efflux at 1500 μm from the root cap junction. Endogenous ABA content, basipetal auxin transport and H^+ efflux in Uod-7 plants under moderate water stress are plotted as 100%. The values are the means and SDs of six replicates from two independent experiments. A ranking test was performed for a, b or c in figures, and different letters (above bars) indicate significant difference at the $P < 0.05$ level.

transport in the root tip (Sharp, 2002; Souter *et al.*, 2002; Ruzicka *et al.*, 2007). Our results suggest that interactions among ABA, auxin and ethylene are integrated in maintaining root growth under water stress.

In conclusion, our results suggest that ABA accumulation modulates auxin transport in the root tip, which enhances proton secretion for maintaining root growth under moderate water stress. Using mutants of rice (monocotyledonous model plant) and Arabidopsis (dicotyledonous model plant), we found that moderate water stress first increases root-tip ABA accumulation, and that the ABA signalling then modulates the auxin transport in the root apex. After that, transported auxin activates the plasma membrane H^+-ATPase to release more protons in the root tip. This activation is essential in maintaining primary root elongation and root hair development under moderate water stress.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Elongation rate of primary roots of rice or wildtype Arabidopsis (Col-0) under water stress.

Fig. S2 Elongation rate of primary roots in rice or wildtype Arabidopsis (Col-0) exposed to different concentrations of ABA.

Fig. S3 Transcript abundance of *AUX1* or *PIN2* in the root tip (0–5000 μm from the root cap junction) of wildtype Arabidopsis plants (Col-0) under moderate water stress or exogenous ABA treatment.

Fig. S4 Distribution and abundance of auxin or auxin transport carrier (AUX1 or PIN2) in the root tip of wildtype Arabidopsis under moderate water stress or exogenous ABA treatment.

Fig. S5 Root tip adaptation of different Arabidopsis natural accessions under moderate water stress.

Fig. S6 Root tip response of rice plants under moderate water stress.

Table S1 Gene-specific primers used for PCR

Table S2 Absolute data of controls (rice or Arabidopsis), which are expressed as 100% in Figs 3–7

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