Journal of Plant Physiology 168 (2011) 1249–1255

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

Net sodium fluxes change significantly at anatomically distinct root zones of rice (*Oryza sativa* L.) seedlings

Qingyuan Zhou[a,1], Li Wang[a,b,1], Xia Cai[c], Di Wang[b], Xuejun Hua[a], Leqing Qu[a], Jinxing Lin[a], Tong Chen[a,*]

[a] *Key Laboratory of Photosynthesis and Molecular Environmental Physiology, Institute of Botany, Chinese Academy of Sciences, Xiangshan, Beijng 100093, China*
[b] *College of Horticulture and Plant Protection, Yangzhou University, Yangzhou 225009, China*
[c] *Key Laboratory of Resource Biology and Biotechnology in Western China (Northwest University), Ministry of Education, Xi'an 710069, China*

ARTICLE INFO

Article history:
Received 18 May 2010
Received in revised form 19 January 2011
Accepted 19 January 2011

Keywords:
Casparian bands
Different root zones
Na^+ flux
Rice

ABSTRACT

Casparian bands of endodermis and exodermis play crucial roles in blocking apoplastic movement of ions and water into the stele of roots through the cortex. These apoplastic barriers differ considerably in structure and function along the developing root. The present study assessed net Na^+ fluxes in anatomically distinct root zones of rice seedlings and analyzed parts of individual roots showing different Na^+ uptake. The results indicated that anatomically distinct root zones contributed differently to the overall uptake of Na^+. The average Na^+ uptake in root zones in which Casparian bands of the endo- and exo-dermis were interrupted by initiating lateral root primordia (root zone III) was significantly greater than that at the root apex, where Casparian bands were not yet formed (root zone I), or in the region where endo- and exo-dermis with Casparian bands were well developed (root zone II). The measurement of net Na^+ fluxes using a non-invasive scanning ion-selective electrode technique (SIET) demonstrated that net Na^+ flux varied significantly in different positions along developing rice roots, and a net Na^+ influx was obvious at the base of young lateral root primordia. Since sodium fluxes changed significantly along developing roots of rice seedlings, we suggest that the significantly distinct net Na^+ flux profile may be attributed to different apoplastic permeability due to lateral root primordia development for non-selective apoplastic bypass of ions along the apoplast.

Introduction

Plants have developed various strategies and mechanisms to resist salinity stress, in which both anatomical and physiological adaptations of roots play key roles under unfavorable environmental conditions (Lazof and Cheeseman, 1986; Kronzucker et al., 2006). Previous studies have shown that the endodermis of all vascular plants and the exodermis of many angiosperms develop Casparian bands that are deposited in the transverse and the radial walls (Peterson and Lefcourt, 1990; Enstone and Peterson, 1997). The major role of this specialized structure is to block the non-selective apoplastic bypass flow of ions and water through the cortex into the stele of the root (Yadav et al., 1996). However, Casparian bands of the endo- and exo-dermis are not a perfect barrier to apoplastic fluxes of water, dissolved solutes and ions, as well as apoplastic tracer dyes (Steudle et al., 1993; Lux et al., 2004).

Rice is a very salt-sensitive crop species and damage from salinity on rice seedlings may result in an excess transport of NaCl through the root system to the leaves. It has been demonstrated that the apoplastic pathway (also termed as the transpirational bypass flow) is of major importance in the uptake of Na^+ in rice (Yeo et al., 1999; Gong et al., 2006), but the apoplastic barriers differ considerably in structure and function along individual developing roots (Schreiber et al., 1999). With regard to rice, the developing roots can be divided into three anatomically distinct regions in terms of development of Casparian bands (Ranathunge et al., 2003). The first is close to the root apex, where no exo- or endo-dermal Casparian bands have formed. The second is farther from the apex, where Casparian bands of either the endo- or exo-dermis, or both, are mature. The third is even farther from the apex, where lateral root primordia are initiated and Casparian bands are interrupted by developing lateral roots. Although these anatomical differences may affect apoplastic permeability to water and ions, few investigations have dealt with the analyses of changes in permeability along different root zones (Ranathunge et al., 2005). Therefore, it is necessary to quantify the relative contribution of anatomically distinct root regions to the overall net fluxes.

Transport of ions into and out of plant roots has been studied using chemical analysis and tracer techniques (Yeo et al., 1987;

Abbreviations: Ae, aerenchyma; Co, cortical cells; En, endodermis; Ex, exodermis; OPR, the outer part of roots; Rh, Rhizodermis; Scl, sclerenchyma.

* Corresponding author. Tel.: +86 10 62836206; fax: +86 10 62836211.
E-mail address: chentong@ibcas.ac.cn (T. Chen).

[1] These authors contributed equally to this manuscript.

0176-1617/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jplph.2011.01.017

Newman, 2001). Previous studies using microelectrodes to measure ion fluxes showed high spatial and temporal variability along roots in many plant species for a range of ions (Shabala et al., 1997; Colmer and Bloom, 1998), though this approach has its limitations, such as relatively poor selectivity for some specific ions and its omission of unidirectional fluxes, among others (Chen et al., 2005). Recent developments in the scanning ion-selective electrode technique (SIET) allow for the non-invasive measurement of ion fluxes at the root surface of an intact plant at high spatial and temporal resolution (Shabala et al., 1997, 2003). This technique greatly facilitates the quantification of net Na^+ flux along the root surface and will extend our understanding about the mechanism for Na^+ intake during stress conditions. The aim of this study was to assess net Na^+ fluxes and shoot Na^+ content by means of SIET analysis and atomic absorption spectrometry to elucidate the contribution of net sodium fluxes at anatomically distinct root zones of rice seedlings and to provide data for further interpretation of roles of Casparian bands in sodium fluxes.

Materials and methods

Plant materials

Seeds of rice (*Oryza sativa* L. cv. Nipponbare) were germinated for 5 days on wet filter paper in the light at $27 \pm 2\,^{\circ}C$. Seedlings were transferred to an aerated hydroponic culture system and grown in the same growth chamber in which all of the following experiments were conducted, with 12-h photoperiod of 400 $\mu mol\,m^{-2}\,s^{-1}$ photosynthetically active radiation, a relative humidity of 70%, and day-time/night-time temperatures of $27 \pm 2\,^{\circ}C$ and $20 \pm 2\,^{\circ}C$, respectively. The nutrient solution was replaced every 5 days with a solution adjusted to the chamber temperature. The solution contained the macronutrients 0.09 mM $(NH_4)_2SO_4$, 0.05 mM KH_2PO_4, 0.05 mM KNO_3, 0.03 mM K_2SO_4, 0.06 mM $Ca(NO_3)_2$, 0.07 mM $MgSO_4$, 0.11 mM Fe-EDTA and the micronutrients 4.6 μM H_3BO_3, 1.8 μM $MnSO_4$, 0.3 μM $ZnSO_4$ and 0.3 μM $CuSO_4$, pH 5.5–6.0.

Root anatomy and microscopy

Fresh, freehand cross sections were prepared from adventitious roots of 15-day-old rice seedlings. Cross sections were prepared at different distances from the root tip (3, 6, 10, 12, 15, 18, 21, 25, 30, 35, 40, 50, 60, 80, 90, 100, 120, 150, 180, 200, 250 mm, respectively). Subsequently, sections were stained with Sudan III to localize suberin deposited within the cell wall following Gerlach (1984) using a saturated solution of Sudan III (Merck) dissolved in ethanol/water (1:1; v/v). To examine Casparian bands in root exodermal and endodermal cells, sections were stained with 0.1% berberine hemisulphate for 1 h and with 0.5% (w/v) aniline blue for another 1 h (Brundrett et al., 1988). Then, the stained sections were observed under an epifluorescence microscope (Q500 IW, Carl Zeiss, Göttingen, Germany) with UV illumination using the Zeiss filter set 04 (exciter filter BP 450–490 nm, chromatic beam filter FT 510 nm, and barrier filter LP 515 nm). To check for suberin lamellae, sections were stained for 1 h with Fluorol Yellow 088 (Brundrett et al., 1991). Then the stained sections were observed under the epifluorescence microscope using an ultraviolet filter set (excitation filter BP365, dichroitic mirror FT 395, emission 397; Carl Zeiss). The method of Hacket and Stewart (1969) was used to determine the position of lateral root primordia in the axes of roots.

Measurement of Na^+ contents from different zones along roots

For each 15-day-old rice seedling, five adventitious roots were selected for experiments; the remaining roots were excised at the basal node of the stem and cut ends were sealed with molten wax. The selected individual roots were examined under a binocular microscope and divided into three zones. Zone I represented the growing tip (0–20 mm from the apex), where both exodermis and endodermis were not mature yet; zone II was the part proximal to zone I (40–60 mm from the apex), where either endodermis or exodermis or both were developed; and zone III was the basal root part, where lateral root primordia were initiated and lateral roots had emerged from the parent root.

We made a plastic box (15 cm long and 12 cm wide, divided into three compartments by two septa, with 5 pores at the base of each septum, 1 cm between pores and pore diameter of 1.5 mm) for the seedlings so that we could measure net fluxes at the three distinct root zones. To examine net fluxes in zone I, this root zone was allowed to penetrate pores of the septum into the middle compartment of the box, which contained 25 mM NaCl culture solution. The remaining parts of the root were contained within the end compartment without NaCl. Leakages of septa were sealed with silicon gel to ensure that solution flow between compartments was prohibited. Root zone I was allowed to take up Na^+ for 48 h, after which the solution in the middle compartment was replaced by culture solution for a chase period of 48 h to carry any Na^+ in the root xylem through to the shoot. The whole shoot was harvested and the Na^+ content determined by atomic absorption spectrometry (model 5100 PC, PerkinElmer, Norwalk, CT, USA).

To measure net Na^+ content of root zone II, root zone I was sealed with silicon gel and zones I and II were bathed in 25 mM NaCl solution in the middle compartment of the box, while zone III was bathed in the end compartment without NaCl. Root zone II was also allowed to take up sodium for 48 h, after which the solution in the middle compartment was replaced by culture solution for a chase period of 48 h to carry any Na^+ in the root xylem through to the shoot. The whole shoot was harvested and Na^+ content was determined by atomic absorption spectrometry.

For examination of root zone III, zones I and II were bathed in the middle compartment of the box containing culture solution without NaCl, and zone III was bathed in 25 mM NaCl in the end compartment. Root zone III was allowed to take up Na^+ for 48 h, after which the solution in the middle compartment was replaced by culture solution for a chase period of 48 h to carry any Na^+ in the root xylem through to the shoot. The whole shoot was harvested and Na^+ content determined by atomic absorption spectrometry. The data were statistically analyzed by using the Student's *t*-test. Significant differences in Na^+ uptake amounts ($P < 0.05$) among the three root zones were assessed by SPSS 12.0 (SPSS Inc., Chicago, IL, USA) using one-way ANOVA analysis.

Na^+ fluxes measurement with the non-invasive scanning ion-selective electrode technique

Net Na^+ fluxes were measured by Xu Yue (Beijing) Science and Technology Co., Ltd. using the non-invasive scanning ion-selective electrode technique (SIET, BIO-IM, Younger USA LLC, Amherst, MA 01002, USA). The microelectrodes were backfilled with 250 mM NaCl solution to a length of approximately 1 cm from the tip, then were front-filled with approximately 25 μm columns of selective liquid ion exchange (LIXs; Na^+, 71178, Sigma–Aldrich, Louis, MO 63103, USA). Prior to fluxes measurement, the microelectrode was calibrated with different concentrations of Na^+ buffer (0.3 mM, 0.9 mM and 3 mM). Only electrodes with Nernstian slope >50 mV/decade were used in our study. Ion flux was calculated by Fick's law of diffusion: $J = -D\,(dc/dx)$, where J is the ion flux in the x direction, dc is the ion concentration difference, dx is the distance of microelectrode movement between two points and D is the ion diffusion coefficient in a particular medium (diffusion coefficient of Na^+ is $2.86 \times 10^{-5}\,cm^{-2}\,s^{-1}$ at $25\,^{\circ}C$).

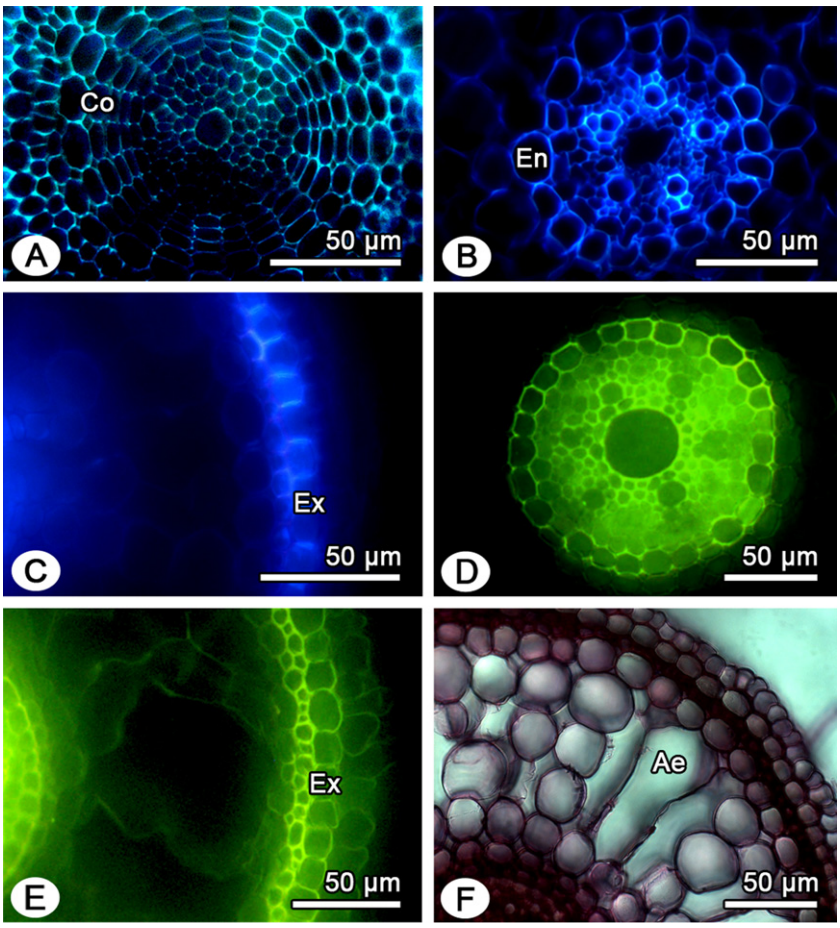

Fig. 1. Freehand cross-sections of rice roots. Panels A–E were viewed under an epifluorescence microscope. Panel F was viewed under bright-field illumination. (A) Part of a cross-section of the apical region of roots, which was taken at a distance of 15 mm from the root tip where the endodermis was immature, stained with aniline blue. (B) Endodermal cell walls with Casparian bands were shown in root zone II, 25 mm behind the root tip. (C) Exodermal cell walls with Casparian bands were shown in root zone II, 30 mm behind the root tip. (D) Endodermal suberin lamellae were well-developed at 50 mm behind the root tip, stained with Fluorol Yellow 088. (E) Exodermal suberin lamellae were well developed at about 60 mm. (F) Cortical cells started to collapse and aerenchyma gradually formed at a distance of 25 mm from the root tip, stained with Sudan III. Bar = 50 μm.

The rice roots were incubated in the measuring solution (0.1 mM KCl, 0.1 mM $CaC1_2$, 0.1 mM $MgC1_2$, 0.5 mM NaCl, 0.2 mM Na_2SO_4, 0.3 mM MES, pH 6.0, adjusted with Tris) to equilibrate for 30 min. Roots were then transferred to the Petri dish containing 10 mL of fresh measuring solution. As a control experiment, the rice seedlings were pre-treated with 100 μM amiloride (Na^+/H^+ antiporter inhibitor) for 30 min in order to inhibit the plasma membrane transporters. Net Na^+ fluxes measurements commenced from the root tip and were repeated along the length of the root at various positions. The stable ion fluxes were recorded for about 15 min at each position. The microelectrode oscillated with an excursion of 30 μm, completing a whole cycle in 5.36 s. Ion fluxes (pmol cm^{-2} s^{-1}) were calculated using MageFlux software, developed by Xu Yue (http://xuyue.net/mageflux). The data were statistically analyzed by the Student's *t*-test.

Results

Structural changes along the length of rice roots

As shown in Fig. 1, the cortical cells of apical regions of rice roots exhibited little or very little morphological specialization and Casparin bands were not detected (Fig. 1A). A primary endodermis with Casparian bands in the radial cell wall was detected at about 25 mm from the root tip (Fig. 1B). Casparian bands in the exodermis started to form somewhat later than those in the endodermis (about 30 mm from the root tip; Fig. 1C). Endodermal suberin lamellae first appeared 35 mm behind the root tip and were fully developed at about 50 mm (Fig. 1D). Exodermal suberin lamellae started to develop about 35 mm from the tip and were well-developed at about 60 mm (Fig. 1E). Patchy development of the exodermis was not found, and maturation of Casparian bands in the exodermis was fairly uniform along the roots.

At a distance of 25 mm from the root tip, cortical cells started to collapse and be enlarged, and air-filled spaces (aerenchyma) gradually formed along roots as a result of cell lysis (Fig. 1F). A fully developed aerenchyma was found at about 120 mm from the root tip (Fig. 2A). Aerenchyma in the central cortex caused separation of the outer part of the root (OPR) from the inner part. A well-defined OPR consisted of four discrete cell layers, i.e. the outermost rhizodermis, exodermis (hypodermis with Casparian bands), a layer of sclerenchyma and the innermost unmodified cortical cell layer (Fig. 2B).

Initiation of lateral root primordia was observed at about 60 mm from the tip (Fig. 2C). Lignified and suberised exodermis and sclerenchymatous cells were not found in the position opposite to the developing lateral root primordia; the cylinder of the endodermis was not continuous, as the lateral root primordia were formed from the pericycle of the parent root (Fig. 2D). Lysigenous aerenchyma channels were never detected in the immediate vicinity of lateral root primordia.

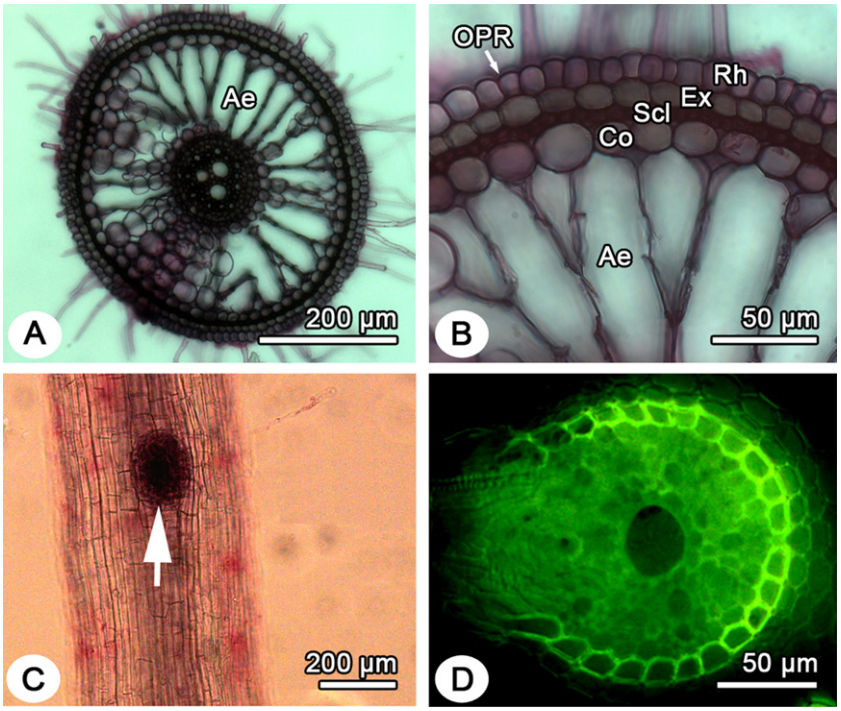

Fig. 2. Lysigenous aerenchyma channels and lateral root primordium of rice roots. Panels A–C were viewed under bright-field illumination. Panel D was viewed under an epifluorescence microscope. (A) Well-developed aerenchyma of roots was observed at about 120 mm from the root tip. (B) Well-defined OPR (the outer part of roots) comprised rhizodermis, exodermis, sclerenchyma and one central cortical cell layer. (C and D) A lateral root primordium was formed from the stele of the parent root. C, from a clearing material of roots, D, from an anatomical section of roots. Bars: A and C = 200 μm; B and D = 50 μm.

Na^+ transport along the length of rice roots

Atomic absorption spectrometry was employed to examine relative Na^+ contents in three anatomically distinct zones of rice roots of the same amount. Considerable variation in individual Na^+ content was observed in the plants from different treatments. Shoot Na^+ content in plants taking up Na^+ only in root zone I (treatment I), zone II (treatment II) or zone III (treatment III) ranged from 0.02 to 0.16 μmol (Fig. 3A), 0.01 to 0.23 μmol (Fig. 3B) and 0.06 to 3.10 μmol (Fig. 3C), respectively, while the average shoot Na^+ contents in the plants from treatments I, II and III were 0.092 ± 0.048 μmol, 0.074 ± 0.067 μmol and 1.382 ± 0.911 μmol, respectively (Fig. 3D). The average Na^+ transport per millimeter root length in zone I $(0.612 \pm 0.342) \times 10^{-2}$ μmol/mm was higher than that in zone II $(0.491 \pm 0.251) \times 10^{-2}$ μmol/mm. The average Na^+ transport per millimeter root length was 0.012 ± 0.007 μmol/mm in zone III, which was significantly higher than those in root zones I and II (Table 1).

Net Na^+ flux measurements using SIET along the length of rice roots

To examine the dynamic Na^+ flow at anatomically distinct zones corresponding to different developing statuses in terms of Casparian band development, net fluxes of Na^+ were measured by using the non-invasive scanning ion-selective electrode technique at anatomically distinct zones (the root apex, the zone that corresponded to the site where Caparian bands have been developed, and the zone of lateral root initial development) along developing roots of 15-day-old rice seedlings. The SIET data showed that the dynamic patterns of net Na^+ fluxes changed significantly in the anatomically distinct zones along the entire length of the rice roots (Fig. 4). A slight net Na^+ influx was observed in the region about 0–300 μm from the root tip (apex zone), where Casparian bands had not been developed yet. Sodium fluxes showed a tendency for fast oscillations in the zone that corresponded to the site where the Caparian strip had been developed, which were comparable to the background recording. These results can be interpreted as a reflection of the temporal behavior in this specific area as suggested by previous studies (Shabala and Knowles, 2002). The net sodium fluxes in zone I (which largely fluctuated within 0 to +100 pmol/cm/s) and zone II (which largely fluctuated within −50 to +50 pmol/cm/s) were not significantly different from those in the background recordings (which fluctuated within a −50 to +50 pmol/cm/s range) in terms of the altitude of fluctuation. Interestingly, a net Na^+ influx was frequently detected at the zone of the initial lateral root development, which was significantly higher than that detected in the root apex (Fig. 4). This kind of net Na^+ influx corresponded to the site where Casparian bands were interrupted by the development of lateral root primordia from the pericycle of the parent root.

In order to further confirm that the selectivity of the specific Na^+ LIX was sufficient for Na^+ flux detection, we have applied a range-specific inhibitor affecting Na^+ fluxes across the plasma membrane (namely amiloride, a Na^+/H^+ exchanger blocker, at a concentration of 100 μM) for pre-treatment of the rice seedlings for 30 min. The results showed that the net Na^+ fluxes significantly decreased in comparison to those in the control plants after the Na^+/H^+ exchanger was inhibited by amiloride treatment. This suggests that the detected signals were largely carried by Na^+ across the plasma membrane (Fig. 5) and the selectivity of LIX was sufficient for our SIET detection in the present study.

Discussion

In the plant body, the apoplast represents an extraprotoplastic compartment, which consists of the cell wall, gas- or water-filled intercellular spaces and the xylem. Accumulating evidence has shown that free movement of water and ions through the apoplast of roots may be hampered by the presence of Casparian bands and

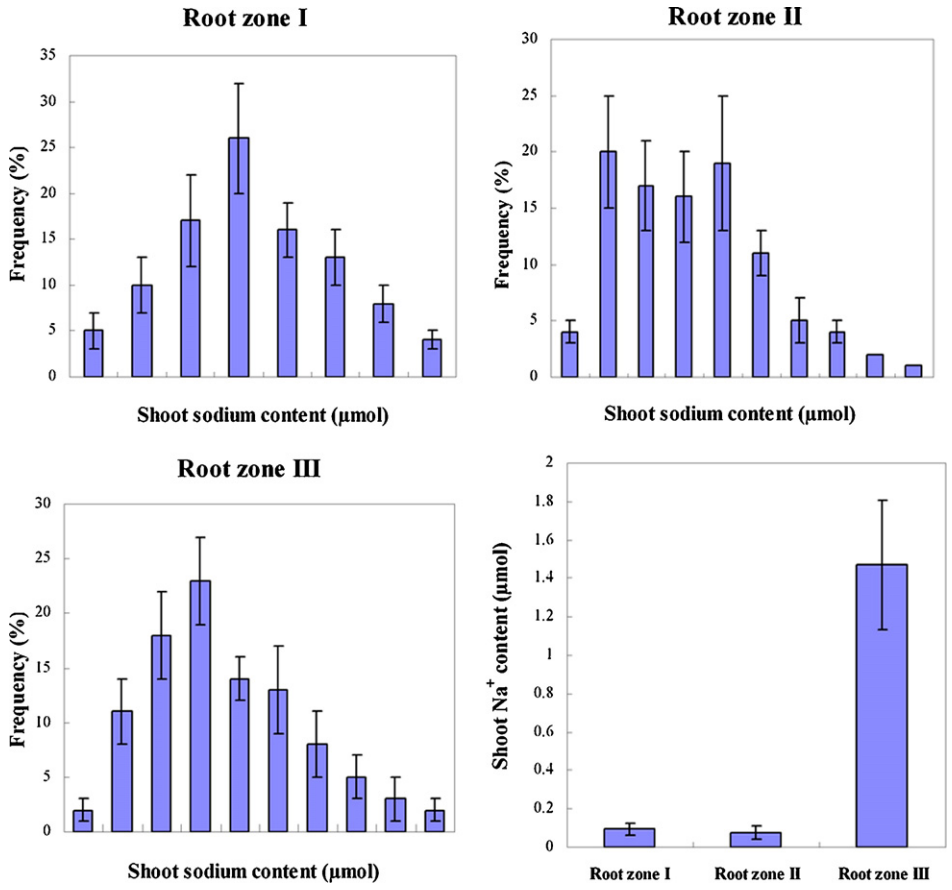

Fig. 3. Frequency distribution of shoot sodium content in plants under different treament conditions and comparisons of Na^+ transport between the three root zones of rice seedlings. (A) plants that were allowed to take up sodium only at the root zone I; (B) plants that were allowed to take up sodium only at the root zone II; (C) plants that were allowed to take up sodium only at the root zone III. The *x*-axis is the quantity of sodium (μmol) in the shoot and the figures are for the upper boundary of the class interval; (D) showing shoot Na^+ content in plants of treatments I, II and III, means ± standard error ($n = 50$).

suberin lamellae in the endo- and exo-dermis (Zeier and Schreiber, 1998; Schreiber et al., 2005). Colmer and Bloom (1998) also suggested that the sclerenchymatous layer may pose a barrier to ion uptake in rice roots, and the degree of lignification of the cell wall in the sclerenchymatous layer may determine the ion absorption capacity along the entire root. Through the analysis of anatomical structure along the different zones, we revealed that Casparian bands in the endo-/exo-dermis started to form at a position about 25–30 mm from the root tip and suberin lamellae started to develop at about 35 mm from the root tip. Furthermore, our anatomical data showed that a region of the exodermis opposite to developing lateral root primordia lacked impregnation with lignin and suberin,

Table 1
Na^+ transport along the length of rice roots.

	Sum of squares	df	Mean square	*F*	Sig.
ANOVA					
Between root zones	65.945	2	32.973	285.261	0.000
Within root zones	17.223	149	.116		
Total	83.168	151			

	(I) Zones	(J) Zones	Mean difference (I – J)	Std. error	Sig.
Multiple comparisons					
LSD	Root zone I	Root zone II	.0193	0.0681	0.778
		Root zone III	−1.378[a]	0.0681	0.000
	Root zone II	Root zone I	−.0193	0.0681	0.778
		Root zone III	−1.398[a]	0.0667	0.000
	Root zone III	Root zone I	1.378[a]	0.0681	0.000
		Root zone II	1.398[a]	0.0667	0.000
Games-Howell	Root zone I	Root zone II	.0193[a]	0.0069	0.018
		Root zone III	−1.378[a]	0.0805	0.000
	Root zone II	Root zone I	−.0193[a]	0.0069	0.018
		Root zone III	−1.398[a]	0.0805	0.000
	Root zone III	Root zone I	1.378[a]	0.0805	0.000
		Root zone II	1.398[a]	0.0805	0.000

[a] The mean difference is significant at the 0.05 level.

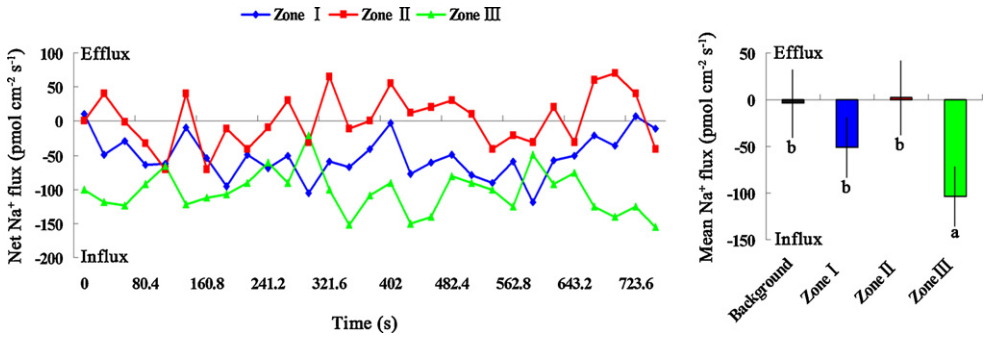

Fig. 4. Net fluxes of Na^+ along three anatomically different zones along intact rice adventitious roots. Among them, zone1, zone 2 and zone 3 corresponded to root apex, the site where Caparian strip had been developed and zone of lateral root initial development respectively. Each point on the diagram represented the mean ± standard error of three replicates, with each replicate being a separated experiment on an individual plant. The mean flux values during the measuring periods were shown in the right panels (columns labelled with different letters were significantly different at $P < 0.05$).

and sclerenchymatous cells were missing from this area. Based on the these anatomical results, we speculated that the regions of discontinuity in endodermal Casparian bands created during the development of lateral root primordia from the pericycle may allow significant apoplastic leakage of ions from the external medium into root tissues.

Permeability of apoplastic barriers to water or solute transport has been reported to be related to the amount and chemical composition of aliphatic and aromatic suberins (Schreiber et al., 1999; Zimmermann et al., 2000). It was reported that the growth environment could affect the amount and proportion of suberin in apoplastic barriers, by which plants can effectively regulate efficient uptake of water and solutes by regulating the amount of apoplastic barriers and their chemical composition (Hose et al., 2001). Krishnamurthy et al. (2009) found that more suberin deposited in soil-grown rice roots than those in hydroponically-grown roots, which might be responsible for the complete clogging of fine inter-microfibrillar spaces in cell walls. They also showed that Casparian bands of the endo- and exo-dermis developed much closer to the root tip in salt-stressed rice plants than in the controls. Our results from atomic absorption spectrometry demonstrated that the average Na^+ contents in root zones I and II were significantly lower than those in zone III, which corresponded to positions where lateral roots develop from the pericycle of adventitious roots and the continuity of endo- and exo-dermis was interrupted. These results suggested that the differences in the amount of Na^+ entering the shoot was strongly influenced by the location, amount and chemical composition of apoplastic barriers in three anatomically distinct zones along developing rice roots.

The futile cycling of sodium at the plasma membrane in root cells under unfavorable conditions is normally attributed to sophisticated activities of distinct transport proteins on the plasma membrane (Cheeseman, 1982; Britto and Kronzucker, 2006), in which the majority of Na^+ influx into the plant cell may occur via nonselective cation channels (Essah et al., 2003; Malagoli et al., 2008), while Na^+ efflux appears to be mediated by Na^+/H^+ exchange (Tester and Davenport, 2003; Munns and Tester, 2008; Britto and Kronzucker, 2009). In the roots of corn and broad bean, Peterson et al. (1981) found that the entry point of tracers into primary roots was along the margins of newly emerged secondary roots. Based on their studies in which insoluble inorganic salts precipitated and clogged apoplastic pores of living root tissue, Ranathunge et al. (2005) showed that the local disruption of exodermis during secondary root development from the pericycle could facilitate high apoplastic bypasses in mature rice roots. In the present study, we found that that a slight net Na^+ influx was observed in the region 0–300 μm from the root tip and a significantly higher net Na^+ influx can be frequently detected at the zone of lateral root initial development, while sodium fluxes showed a tendency for fast oscillations in the zone that corresponded to the site where the Caparian strip has been developed, indicating that lateral root primordia may allow significant Na^+ permeation from the apoplastic barriers of the endo-/exodermis. This kind of anatomical characteristic contributes significantly to the overall net Na^+ flux profile and may offer opportunities for ion entry from the external medium into the stele of the root, which were in accordance with the results from a previous report by Flowers and Yeo (1981). In addition, we also noticed that sodium fluxes showed a tendency for fast oscillations in the zone that corresponded to the site where the Caparian strip has been developed, which were comparable to the background recording. These results can be interpreted as a reflection of temporal behavior in this specific area as suggested

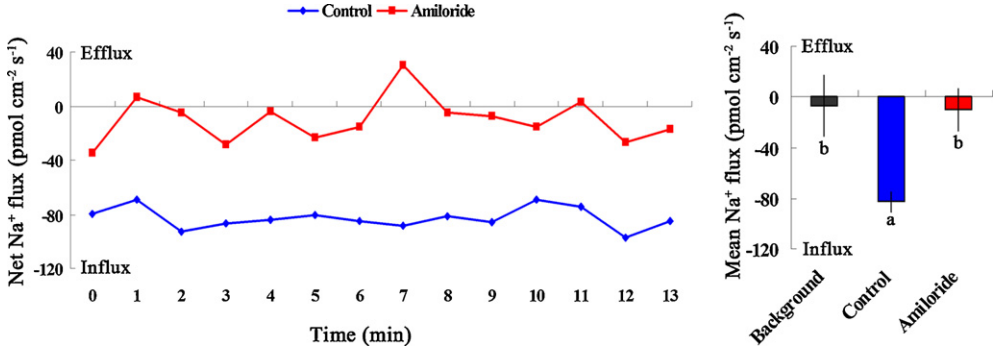

Fig. 5. Effects of pharmacological treatment with 100 μM amiloride on Na^+ flux profiles in the zone of lateral root initial development. The rice seedings were pretreated with 100 μM amiloride (a specific Na^+/H^+ exchanger blocker) for 30 min. Steady-state flux profiles of Na^+ was examined by a continuous flux recording (10–15 min). Each point on the diagram was the mean of 3–5 individual seedlings, and bars represented the standard error of the mean. The mean flux values during the measuring periods were shown in the right panels (columns labelled with different letters were significantly different at $P < 0.05$).

by previous studies (Shabala et al., 1997; Shabala and Knowles, 2002), which may be also partly attributed to the anatomical features of Casparian bands in this zone. The accumulation of Na^+ in roots detected in our study, therefore, should be interpreted as a balance between influx through ion channels and efflux through probable transporters or antiporters. As Casparian bands begin to form at about 25 mm from the root tip (zone II) while the cytoplasm in the cells at the root tip is rather dense (zone I), sodium influxes may be difficult to take place in zone I and zone II but can easily enter the stele at the base of young lateral root primordia (zone III). Since the composition of suberin and lignin as well as the density of Casparian bands were related to the efficiency of the apoplastic barrier (Zimmermann et al., 2000), the findings from non-invasive microelectrode detection provide strong evidence for the view that considerable apoplastic flow of Na^+ occurred in the stele (i.e. apoplastic leakage). Transpirational bypass flow made an important contribution to Na^+ uptake, which may be mainly attributed to the significant net Na^+ influxes detected at the zone of lateral root initial development. Nevertheless, it is still difficult to distinguish the apoplastic pathway from the symplastic pathway by using SIET analysis only. Since bypass flow was reported to be the major pathway of Na^+ entry into the shoots in rice (Yeo et al., 1987; Gong et al., 2006), we consider the non-invasive data on shoot Na^+ contents as an indirect index for bypass flow.

In summary, our investigations provided several lines of evidence for the contribution of net sodium fluxes at anatomically distinct root zones of rice seedlings. We found that Casparian bands in the endo-/exo-dermis as well as suberin lamellae started to form behind zone I, and there were discontinued regions at the position where lateral root primordial developed. Atomic absorption spectrometric analysis demonstrated that average shoot Na^+ content in zone III was significantly higher than those in the other two zones. Moreover, it was revealed by non-invasive Na^+ flux detection that significant net Na^+ influx was frequently detected at the zone of initial lateral root development. Based on the net Na^+ flux profile at the base of young lateral root primordia as detected by SIET and anatomical data, we postulate that “open windows” in both Casparian bands and the sclerenchymatous layer caused by development of lateral root primordia are likely responsible for the net Na^+ influxes in the SIET measurement.

Acknowledgements

The authors thank Professor Hu YuXi of Institute of Botany, Chinese Academy of Sciences for his assistance in the preparation of this manuscript, and this work was supported by an International Science and Technology Cooperation Program from Ministry of Science and Technology of China (2007DFA30770) and an international cooperation project from Sino-German Center (GZ616).

References

Britto DT, Kronzucker HJ. Futile cycling at the plasma membrane: a hallmark of low-affinity nutrient transport. Trends Plant Sci 2006;11:529–34.
Britto DT, Kronzucker HJ. Ussing's conundrum and the search for transport mechanisms in plants. New Phytol 2009;183:243–6.
Brundrett MC, Enstone DE, Peterson CA. A berberine-aniline blue fluorescent staining procedure for suberin, lignin, and callose in plant tissue. Protoplasma 1988;146:133–42.
Brundrett MC, Kendrick B, Peterson CA. Efficient lipid staining in plant material with Sudan Red 7B or Fluorol Yellow 088 in polyethylene glycol-glycerol. Biotech Histochem 1991;66:111–6.
Cheeseman J. Pump-leak sodium fluxes in low salt corn roots. J Membr Biol 1982;70:157–64.
Chen Z, Newman I, Zhou M, Mendham N, Zhang G, Shabala S. Screening plants for salt tolerance by measuring K^+ flux: a case study for barley. Plant Cell Environ 2005;28:1230–46.
Colmer TD, Bloom AJ. A comparison of NH_4^+ and NO_3^- net fluxes along roots of rice and maize. Plant Cell Environ 1998;21:240–6.
Enstone DE, Peterson CA. Suberin deposition and band plasmolysis in the corn (*Zea mays* L.) root exodermis. Can J Bot 1997;75:1188–99.
Essah PA, Davenport R, Tester M. Sodium influx and accumulation in *Arabidopsis*. Plant Physiol 2003;133:307–18.
Flowers TJ, Yeo AR. Variability in the resistance of sodium chloride salinity within rice (*Oryza sativa* L.). New Phytol 1981;88:363–73.
Gong HJ, Randall DP, Flowers TJ. Silicon deposition in the root reduces sodium uptake in rice (*Oryza sativa* L.) seedlings by reducing bypass flow. Plant Cell Environ 2006;29:1970–9.
Hacket C, Stewart H. A method for determining the position and size of lateral primordia in the axes of roots without sectioning. Ann Bot 1969;33:679–82.
Hose E, Clarkson DT, Steudle E, Schreiber L, Hartung W. The exodermis: a variable apoplastic barriers. J Exp Bot 2001;52:2245–64.
Krishnamurthy P, Ranathunge K, Franke R, Prakash HS, Schreiber L, Mathew MK. The role of root apoplastic transport barriers in salt tolerance of rice (*Oryza sativa* L.). Planta 2009;230:119–34.
Kronzucker HJ, Szczerba MW, Moazami-Goudarzi M, Britto DT. The cytosolic Na^+:K^+ ratio does not explain salinity-induced growth impairment in barley: a dual-tracer study using $^{42}K^+$ and $^{24}Na^+$. Plant Cell Environ 2006;29:2228–37.
Lazof D, Cheeseman JM. Sodium transport and compartmentation in *Spergularia marina*. Plant Physiol 1986;81:742–7.
Lux A, Scottnikova A, Opatrna J, Greger M. Differences in structure of adventitious roots in *Salix* clones with contrasting characteristics of cadmium accumulation and sensitivity. Physiol Plant 2004;120:537–45.
Malagoli P, Britto DT, Schulze LM, Kronzucker HJ. Futile Na^+ cycling at the root plasma membrane in rice (*Oryza sativa* L.): kinetics, energetics, and relationship to salinity tolerance. J Exp Bot 2008;59:4109–17.
Munns R, Tester M. Mechanisms of salinity tolerance. Annu Rev Plant Biol 2008;59:651–81.
Newman IA. Ion transport in roots: measurement of the fluxes using ion-selective microelectrodes to characterize transporter function. Plant Cell Environ 2001;24:1–14.
Peterson CA, Lefcourt BEM. Development of endodermal Casparian bands and xylem in lateral roots of broad bean. Can J Bot 1990;68:2729–35.
Peterson CA, Emanuel ME, Humphreys GB. Pathway of movement of apoplastic dye tracers through the endodermis at the site of secondary root formation in corn (*Zea mays*) and broad bean (*Vicia faba*). Can J Bot 1981;59:618–25.
Ranathunge K, Steudle E, Lafitte R. Control of water uptake by rice (*Oryza sativa* L.): role of the outer part of the root. Planta 2003;217:193–205.
Ranathunge K, Steudle E, Lafitte R. A new precipitation technique provides evidence for the permeability of Casparian bands to ions in young roots of corn (*Zea mays* L.) and rice (*Oryza sativa* L.). Plant Cell Environ 2005;28:1450–62.
Schreiber L, Hartmann K, Skrabs M, Zeier J. Apoplastic barriers in roots: chemical composition of endodermal and hypodermal cell walls. J Exp Bot 1999;50:1267–80.
Schreiber L, Franke R, Hartmann KD, Ranathunge K, Steudle E. The chemical composition of suberin in apoplastic barriers affects radial hydraulic conductivity differently in the roots of rice (*Oryza sativa* L. cv IR64) and corn (*Zea mays* L. cv. Helix). J Exp Bot 2005;56:1427–36.
Shabala SN, Knowles AE. Rhythmic patterns of nutrient acquisition by wheat roots. Funct Plant Biol 2002;29:595–605.
Shabala SN, Newman IA, Morris J. Oscillations in H^+ and Ca^{2+} ion fluxes around the elongation region of corn roots and effects of external pH. Plant Physiol 1997;113:111–8.
Shabala SN, Shabala L, Van Volkenburgh E. Effect of calcium on root development and root ion fluxes in salinised barley seedlings. Funct Plant Biol 2003;30:507–14.
Steudle E, Murrmann M, Peterson CA. Transport of water and solutes across maize roots modified by puncturing the endodermis. Further evidence for the composite transport model of the root. Plant Physiol 1993;103:335–49.
Tester M, Davenport R. Na^+ tolerance and Na^+ transport in higher plants. Ann Bot 2003;91:503–27.
Yadav R, Flowers TJ, Yeo AR. The involvement of the transpirational bypass flow in sodium uptake by high- and low-sodium-transporting lines of rice developed through intravarietal selection. Plant Cell Environ 1996;19:329–36.
Yeo AR, Yeo ME, Flowers TJ. The contribution of an apoplastic pathway to sodium uptake by rice roots in saline conditions. J Exp Bot 1987;38:1141–53.
Yeo AR, Flowers SA, Rao G, Welfare K, Senanayake N, Flowers TJ. Silicon reduces sodium uptake in rice (*Oryza sativa* L.) in saline conditions and this is accounted for by a reduction in the transpirational bypass flow. Plant Cell Environ 1999;22:559–65.
Zeier J, Schreiber L. Comparative investigation of primary and tertiary endodermal cell walls isolated from the roots of five monocotyledonous species: chemical composition in relation to fine structure. Planta 1998;206:349–61.
Zimmermann HM, Hartmann K, Schreiber L, Steudle E. Chemical composition of apoplastic transport barriers in relation to radial hydraulic conductivity of corn roots (*Zea mays* L.). Planta 2000;210:302–11.