Osmotic Stress and Ion-Specific Effects on Xylem Abscisic Acid and the Relevance to Salinity Tolerance in Poplar

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ABSTRACT

We designed two experiments to investigate the osmotic stress and ion-specific effects on xylem abscisic acid (ABA) and the relevance to salinity tolerance in one-year-old seedlings of *Populus euphratica* Oliv. (a salt-resistant genotype) and one-year-old rooted cuttings of *P. 'popularis 35-44'* (*P. popularis*) (a salt-sensitive genotype). Net photosynthetic rates (Pn) and unit transpiration rates (TRN) of the two genotypes were significantly decreased upon osmotic shock caused by PEG 6000 (osmotic potential = -0.24 MPa) or iso-NaCl (50 mM). Shoot xylem ABA concentrations in both genotypes increased rapidly after the onset of PEG stress, resulting from a decreased water flow. NaCl-treated trees of *P. euphratica* maintained considerably greater concentrations of ABA than PEG-treated plants in a longer term, whereas salinized *P. popularis* exhibited a transient accumulation of ABA in the shoot. TRN was greatly enhanced in both genotypes when pressure (0.24 MPa) was applied to counteract the osmotic suction of 50 mM NaCl. Pressurizing of root systems diluted solutes in the root xylem, but the dilution effect was more pronounced in *P. popularis*. Root xylem ABA concentrations in *P. euphratica* steadily increased with salt stress although pressurization lowered its levels. In contrast, there were no observed changes in ABA response to salinity in pressured *P. popularis*. Therefore, we concluded that the salt-tolerant *P. euphratica* had a greater capacity to synthesize ABA under saline conditions, which may partially result from specific salt effects. In addition, *P. euphratica* exhibited a higher capacity for salt (Na^+ and Cl^-) transport control under salt stress, compared with *P. popularis*. The possible association between ABA and salt transport limitation, and the relevance to salinity tolerance were discussed.

Key words: NaCl; PEG; Xylem ABA; Transpiration; Photosynthesis; Pressurization; *Populus euphratica*; *P. popularis*

INTRODUCTION

There is now considerable experimental evidence suggesting that root-derived abscisic acid (ABA) may function as a modulator of plant response to

salinity. In recent years, salt-induced increase of endogenous ABA has been observed in many plants, for example, *Lupinus* (Wolf and others 1990), barley (Zhao and others 1991), ice plant (Thomas and others 1992), rice (Asch and others 1995; Moons and others 1995), tomato (Dunlap and Binzel 1996; Plant and others 1991), *Brassica* (He and Cramer 1996), castor bean (Jeschke and others 1997), bush bean (Montero and others 1997, 1998) and *Citrus* (Gómez-Cadenas and others 1998). However, attempts to correlate ABA production to salinity tolerance in plants have resulted in apparently conflicting observations. Salt-tolerant rice varieties accumulated more ABA and increased ABA more rapidly than the sensitive cultivar (Moons and others 1995). Normal tomato genotypes showed a greater capacity to accumulate ABA than an ABA-deficient mutant (salt sensitive) (Dunlap and Binzel 1996). Conversely, the levels of salt-induced ABA were negatively correlated with the salt tolerance of *Brassica* species (He and Cramer 1996) and rice cultivars (Asch and others 1995). This negative correlation has also been reported in a study with saltbush, barley, and cotton (Zhao and others 1991). These conflicting studies on a number of plant species indicate different mechanisms of salt adaptation. However, the relationship between ABA and salinity tolerance has not been extensively investigated in poplar (Chen and others 2001).

A transient endogenous ABA accumulation was found to be significantly correlated with osmotic potentials of the root medium (Ribaut and Pilet 1991), suggesting that salt-induced ABA may be triggered by a changed water relation rather than by a specific salt effect (He and Cramer 1996; Zhao and others 1991). However, in a more recent study, Montero and others (1998) suggested that an ion-specific effect was responsible for increased leaf ABA. In a previous report, we observed significantly elevated xylem ABA in *P. euphratica* upon salt stress (Chen and others 2001). However, little information is available on the osmotic stress and ion-specific effects to salt-induced ABA.

In the present study, we attempted to compare the endogenous xylem ABA of a salt-resistant poplar genotype *P. euphratica* with that of a salt-sensitive genotype *P. 'popularis* 35-44' (*P. popularis*) after the onset of osmotic shock or NaCl stress. To alleviate the osmotic stress on shoots, we also applied pressure to the roots of plants to counteract the osmotic suction of external NaCl (for theory see Passioura and Munns 1984). The relative importance of osmotic stress and ion-specific effects on ABA production, and the relevance to salinity tolerance of poplar were discussed.

MATERIALS AND METHODS

Plant Materials

One-year-old seedlings of *Populus euphratica* and one-year-old rooted cuttings of *Populus 'popularis* 35-44' (*P. popularis*) were used in this experiment. In mid-April, seedlings of *P. euphratica*, obtained from Xinjiang Uygur Autonomous Region of China and cuttings of *P. popularis* were planted individually in 5 or 10 L pots containing sand (5 L pots could fit inside pressure chambers). Potted plants were placed in a glasshouse at the Experimental Center of Forest Biology, Beijing Forestry University (China). Plants were kept well watered as required and received 1 L of full strength Hoagland's nutrient solution every 2 weeks. All plants were grown for 2 months prior to the experiment (June 20).

Experiment 1

Stomatal response to osmotic and NaCl stress. Plants established in 10 L pots (0.4–0.6 m high, with 20–30 leaves) were selected in terms of uniformity. The same three treatments were applied in both *P. euphratica* and *P. popularis* at 8:30–9:00 AM: (A) control; (B) NaCl; (C) PEG. Saline treatment was imposed by top watering of 5 L of 50 mM NaCl solution (osmotic potential = −0.24 MPa). For PEG treatment, plants received 5 L of PEG 6000 solution of the same osmotic potential. Control plants were well irrigated with no addition of NaCl or PEG. When the treatments were initiated, gas exchange of upper mature leaves was measured with a Li-6200 portable photosynthesis system (Li-Cor Inc, Lincoln, NE, USA). Air temperature was 26–30°C and irradiance was 1000 μmol m^{-2}s^{-1} photosynthetically active radiation (PAR) supplied by cool white fluorescent lamps supplemented with dysprosium lamps. Following gas exchange measurements, terminal twigs were excised and used for sap collection. The sampling of xylem sap was made after 1, 4, 8, and 24 h of treatments. Four replicated seedlings per treatment were harvested at each sample time.

Shoot xylem sap extraction. Collection of shoot xylem sap has been described in detail elsewhere (Chen and others 1997, 2001) but a brief description is given. The terminal twigs (15 cm long) were excised and immediately enclosed in a pressure chamber. Bark was removed to a height of about 5 cm to avoid xylem sap contamination by phloem extrusion (Fort and others 1997). The pressure was slightly increased above the balancing pressure and then maintained at 0.5 MPa over that pressure for

10 min. Extruded xylem sap was collected with vials and used for ABA analysis.

Experiment 2

Stomatal response to NaCl with or without pressurization. Plants established in 5 L pots (0.2–0.3 m high, with 10–15 leaves) were used in this experiment. *P. euphratica* and *P. popularis* plants were salinized by NaCl with or without pressure. Saline treatment was imposed by top watering of 5 L of 50 mM NaCl solution at 8:30–9:00 AM. For pressurization treatment, pots were placed in pressure chambers, and pressure (0.24 MPa) was immediately applied following the salt treatment. Compressed air was used to pressurize root systems of plants. The pressurization experiment also included plants with no addition of NaCl. When the treatments were initiated, gas exchange was measured with a Li-6200 portable photosynthesis system (Li-Cor Inc, Lincoln, NE, USA) under conditions where air temperature was 26–30°C and PAR was 1000 $\mu mol\ m^{-2}s^{-1}$ supplied by cool white fluorescent lamps supplemented with dysprosium lamps. Four replicated seedlings per treatment were measured at each sample time.

Root xylem sap extraction. Following gas exchange measurements, the stem was cut near its base with a sharp razor blade and bark was removed to a height of about 5 cm. A PVC tube was attached to the stump for root sap collection (Else and others 1994; Passioura 1980). The pressure was slightly increased above the balancing pressure and then maintained at 0.5 MPa over that pressure for 10 min. Extruded root xylem sap was collected and used for ABA and ion analysis. The sampling of xylem sap was made after 1, 4, and 8 h of treatments. Four replicated seedlings per treatment were harvested at each sample time.

Determination of xylem ABA and ion concentrations. Na^+ was quantified by an atomic absorption spectrophotometer (Perkin-Elmer 2280), and Cl^- by silver titration (Chen and others 2001). ABA concentrations in the xylem sap of shoots or roots were determined by HPLC (Chen and Wang 1992). A brief description is given below.

1) ABA purification with reverse-phase HPLC: Following filtration through a layer of $\phi 0.25\ \mu m$ membrane, 100–200 μL sap was injected directly into the chromatograph. HPLC analysis was carried out with a Hitachi-Merck liquid chromatography (L-6200 pump, L-4000 UV detector) using a 4.6 × 250 mm reverse-phase C_{18} column (irregular-H, 5 μm particle size). Acetic acid (100 mmol L^{-1})

containing 20% (v/v) methanol was used as mobile phase at a flow rate of 1.0 ml min^{-1}, and methanol increased up to 100% within 30 min. Detection was performed by UV absorbance at 254 nm. The eluted mobile phase was collected according to the retention time of standard ABA, then the collected eluate (2–3 ml) was dried by vacuum evaporating. Afterwards, the condensate was stored under vacuum in a flask containing P_2O_5 (desiccant) over 24 h (under darkness).

2) ABA identification and quantification with normal HPLC: Normal HPLC was performed with a Hitachi-Merck liquid chromatography (L-6200 pump, L-4000 UV detector) using a Li-Chrospher Si 60 column (4.0 × 152 mm, 5 μm). ABA samples were eluted with a mobile phase containing hexane:iso-propyl alcohol:formic acid 90:9.5:0.5 (v/v) at a flow rate of 1.0 ml min^{-1}. ABA was determined at 254 nm and quantified with a Shimadzu CR4A integrator. All steps of normal HPLC processing were carried out under water-free conditions.

Data analysis. The data were subjected to ANOVA and significant differences between means were determined by Duncan's multiple-range test. Unless otherwise stated, differences were considered statistically significant when $p < 0.05$.

RESULTS

Experiment 1

Gas exchange. Compared with control plants, stomatal conductance (Cs), net photosynthetic rates (Pn), and transpiration rates (TRN) of the two genotypes markedly decreased during the period of NaCl stress (Figure 1). PEG treatment caused a similar effect on gas exchange (Figure 1). In comparison, the inhibitory effects of PEG on Cs and gas exchange were more pronounced in *P. popularis* with the period of exposure whereas in *P. euphratica* it was less than NaCl (Figure 1). Noteworthy, *P. euphratica* maintained typically higher Cs and TRN than *P. popularis* regardless of treatments (Figure 1).

Shoot xylem ABA concentrations. Shoot xylem ABA concentrations in control plants of the two genotypes did not notably change during the period of experiment (Figure 2). ABA in *P. euphratica* abruptly increased to 6.44 mmol m^{-3} after 1 h of PEG treatment, which was 3.3-fold of that in controls (Figure 2). It decreased sharply at 4 h, then increased gradually and reached 2.99 mmol m^{-3} at 24 h (Figure 2). NaCl-treated *P. euphratica* exhibited a marked rise of ABA at 1 h, but the value was
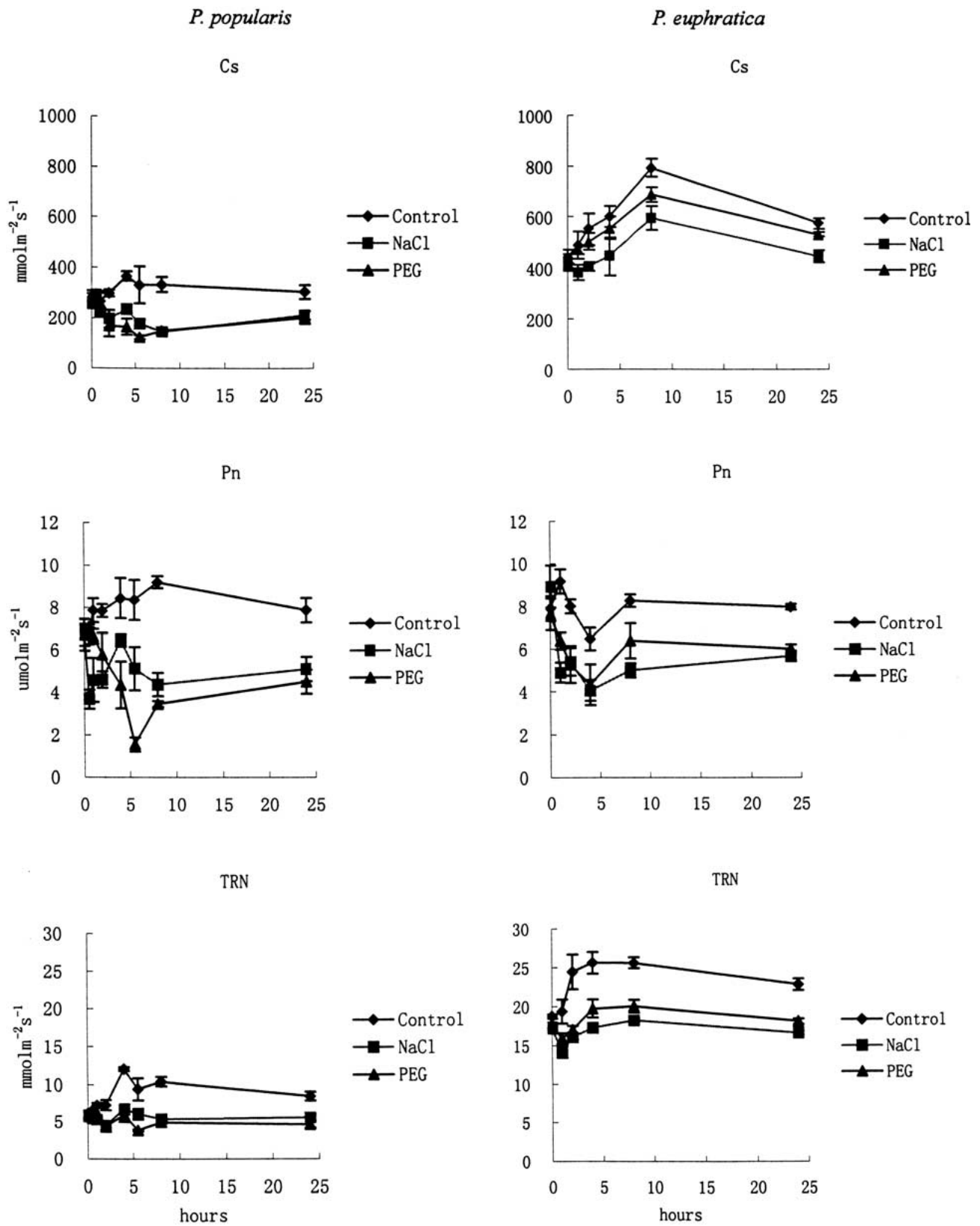
Figure 1. Effect of NaCl and PEG on stomatal conductance (Cs), net photosynthetic rates (Pn), and transpiration rates (TRN) of *P. popularis* and *P. euphratica*. Each point is the mean of four plants and bars represent the standard error of the mean.

P. popularis   P. euphratica
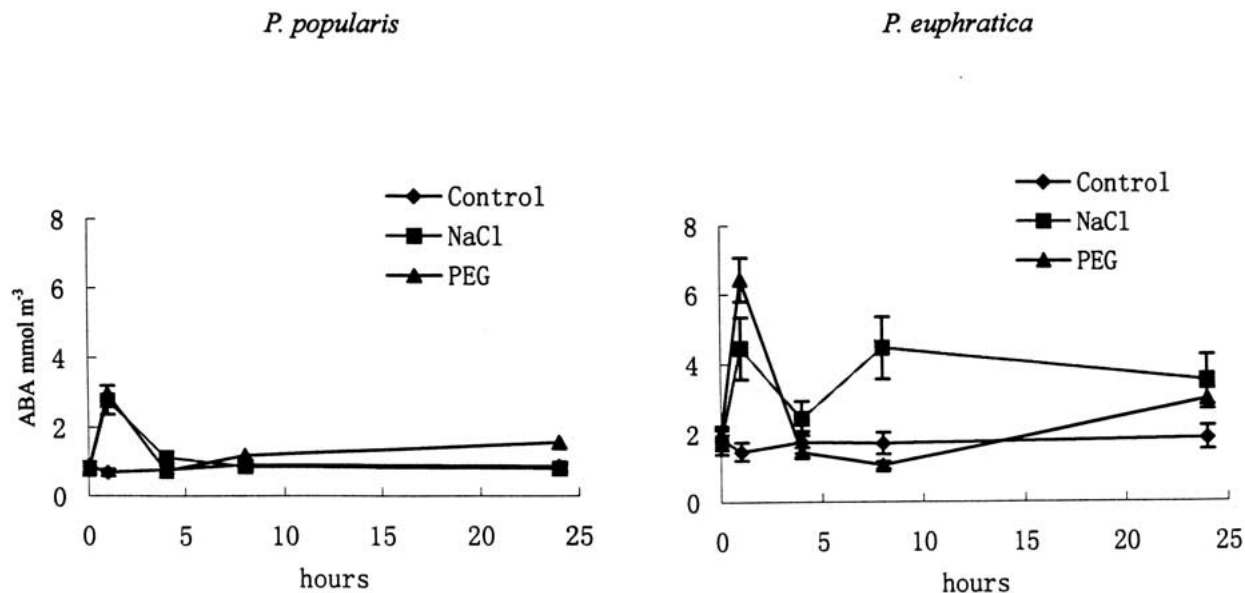
Figure 2. Effect of NaCl and PEG on shoot xylem ABA concentrations of *P. popularis* and *P. euphratica*. Each point is the mean of four plants and bars represent the standard error of the mean.

lower than that induced by PEG (Figure 2). However, salinized plants maintained higher ABA than PEG-treated plants in the following hours, even though a decline was observed at 4 h (Figure 2).

A transient increase of ABA was also observed in PEG-treated *P. popularis* as soon as water stress began, thereafter ABA rapidly dropped to pre-stress levels at 4 h, followed by a gradual increase in the rest of the experiment (Figure 2). NaCl-treated plants of *P. popularis* showed a trend similar to that of PEG-stressed trees, but ABA remained unchanged after it reached the minimum at 4 h (Figure 2).

Experiment 2

Gas exchange. In this experiment, pressurization of the root system did not significantly affect Cs, Pn, or TRN in well-watered plants of either genotype (Figure 3). Stomatal conductance and gas exchange were markedly reduced upon NaCl shock, although *P. euphratica* had typically higher Cs, Pn, and TRN than *P. popularis* regardless of treatments (Figure 3). Notably, pressurization markedly enhanced Cs and TRN of salinized plants compared with those without pressurization (Figure 3). TRN was increased by 13%–24% (*P. euphratica*) and 20%–30% (*P. popularis*), respectively, after 2 h of pressure (Figure 3). However, there were genotypic differences in the Pn response to the applied pressure in salinized plants. Pn of pressurized *P. popularis* followed the same trend as TRN; in contrast, pres-

surization markedly lowered Pn in *P. euphratica* (Figure 3).

Root xylem ABA concentrations. Root xylem ABA concentrations of pressurized *P. euphratica* controls (C + P) did not significantly differ from those of nonpressurized controls (C-P), whereas in *P. popularis*, ABA was significantly reduced by pressurization (Figure 4). Xylem ABA concentrations of nonpressurized plants increased rapidly as soon as salt stress began, but there were genotypic differences in the pattern of ABA response to salinity. *P. euphratica* maintained a typically higher and constant ABA level in the long-term, up to 8 h, whereas *P. popularis* exhibited a transient and lower ABA accumulation (Figure 4). Furthermore, ABA steadily increased in pressurized *P. euphratica* during the period of salt stress, even though pressurization markedly lowered ABA levels compared with those without pressure (Figure 4). In contrast, pressurized *P. popularis* plants showed no observed change of ABA upon salinity (Figure 4).

Sodium and chloride concentrations in the root xylem. Root xylem Na^+ and Cl^- concentrations of *P. popularis* controls were markedly lowered by pressurization, whereas no corresponding change was observed in pressurized *P. euphratica* controls (Figure 5). Na^+ and Cl^- concentrations in nonpressurized *P. popularis* sharply increased after the onset of salt stress and remained at relatively high levels during 8 h of stress, whereas in *P. euphratica* they both started to decline after reaching the maximum at 1 h (Figure 5). Xylem Na^+ and Cl^- of pressurized
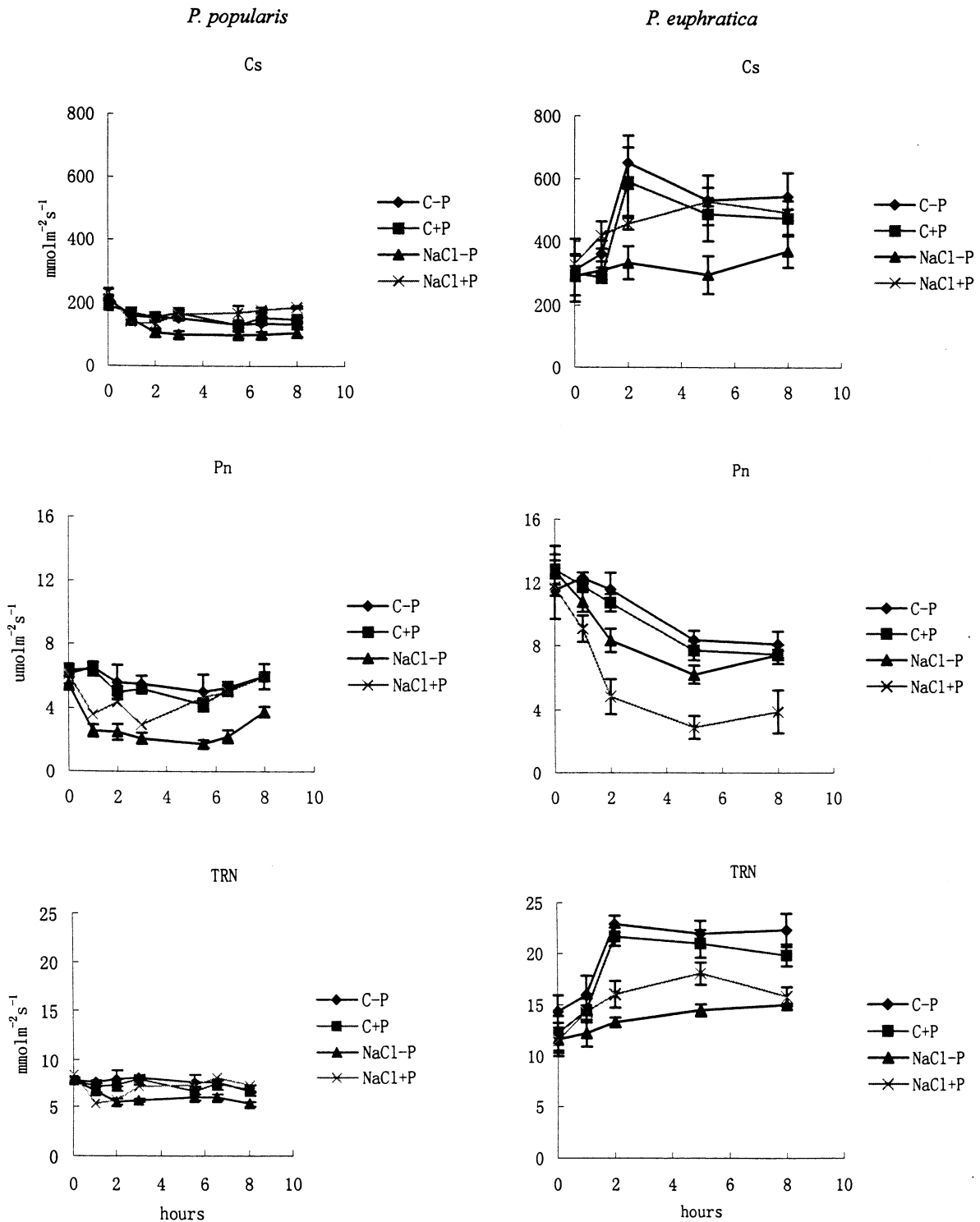
Figure 3. Effect of NaCl and pressurization (P) on stomatal conductance (Cs), net photosynthetic rates (Pn), and unit transpiration rates (TRN) of *P. popularis* and *P. euphratica*. Each point is the mean of four plants and bars represent the standard error of the mean.

P. popularis steadily increased during the period of salt stress, but their levels were lower than those without pressure (Figure 5). In contrast, Na⁺ and

Cl⁻ in salinized *P. euphratica* were enhanced after 8 h of pressurization, despite a slight reduction after the pressure was initiated (Figure 5).

P. popularis
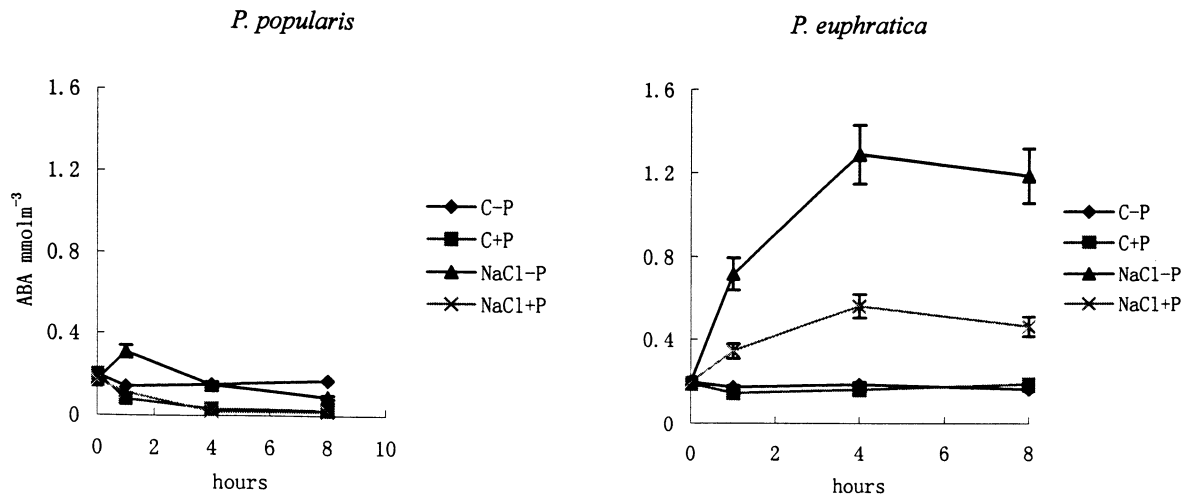
P. euphratica

Figure 4. Effect of NaCl and pressurization (P) on root xylem ABA concentrations of *P. popularis* and *P. euphratica*. Each point is the mean of four plants and bars represent the standard error of the mean.

DISCUSSION

### Osmotic Stress and Ion-Specific Effects on Xylem ABA

A sudden exposure to PEG (osmotic potential was −0.24 MPa) significantly reduced leaf transpiration in the two genotypes (Figure 1), and simultaneously caused a transitory accumulation of ABA in the xylem sap of the shoot (Figure 2). Results suggest that the transient rise of xylem ABA was mainly the result of the imposition of water stress, which caused a decline in water flow, thus concentrating the solute. Similarly, an iso-osmotic NaCl shock (50 mM NaCl, osmotic potential = −0.24 MPa) caused a reduction in water loss (Figure 1), and salinized *P. popularis* accumulated xylem ABA to a magnitude similar to those induced in PEG-treated plants (Figure 2). Accordingly, these results suggest that the initial increase of ABA in NaCl-treated *P. popularis* was mainly the result of a decreased water flow.

In *P. euphratica*, PEG and NaCl both reduced transpiration, and ABA was correspondingly concentrated in the xylem (Figures 1, 2). However, a high ABA value was recorded in the PEG treatment after 1 h of stress (Figure 2). This agrees with a report by Chazen and others (1995) showing that PEG-treated maize plants produced a greater ABA accumulation than those in iso-NaCl, presumably resulting from a high inhibitory effect on water transport in roots. It is noteworthy that salinized *P. euphratica* plants maintained higher ABA levels than PEG-treated plants over a longer term (Figure 2), suggesting that ion-specific effects, in addition to

osmotic stress, contributed to the NaCl-induced ABA (Montero and others 1998).

ABA in PEG-treated plants of the two genotypes showed no marked elevation after more than 4 h of stress (Figure 2), appearing to be the result of oxygen limitation because PEG is known to reduce oxygen availability (Mexal and others 1975). An oxygen limitation could account for the lack of continued ABA production because oxygen is a requirement for ABA synthesis in the root.

When pressure (0.24 MPa) was applied to the roots to counteract the osmotic suction of 50 mM NaCl, leaf transpiration in both genotypes was greatly enhanced (Figure 3). This was the result of pressure-increased radial water flow across the root into the xylem (Liang and Zhang 1997; Munns 1985; Schurr and Schulze 1995). An apoplastic path may be involved for water transport to bypass the cell-to-cell pathway (Steudle and others 1993; Steudle and Meshcheryakov 1996). There were no observed changes of root xylem ABA response to salinity in pressured *P. popularis* (Figure 4). It is suggested that ABA is mostly synthesized in the roots when plants are salinized (Wolf and others 1990; Zhao and others 1991), and pressurization of roots may reduce ABA recirculation from shoots. Therefore, it can be inferred that the ABA synthesis in roots of pressurized *P. popularis* could not keep the pace of water flow, thus causing a decline in xylem ABA. In contrast to *P. popularis* root xylem ABA concentrations in pressurized *P. euphratica* steadily increased upon the NaCl stress and its concentration was less diluted by the increased water flow (Figure 4), indicating an apoplastic bypass flow of ABA. Freundl and others (1998) have
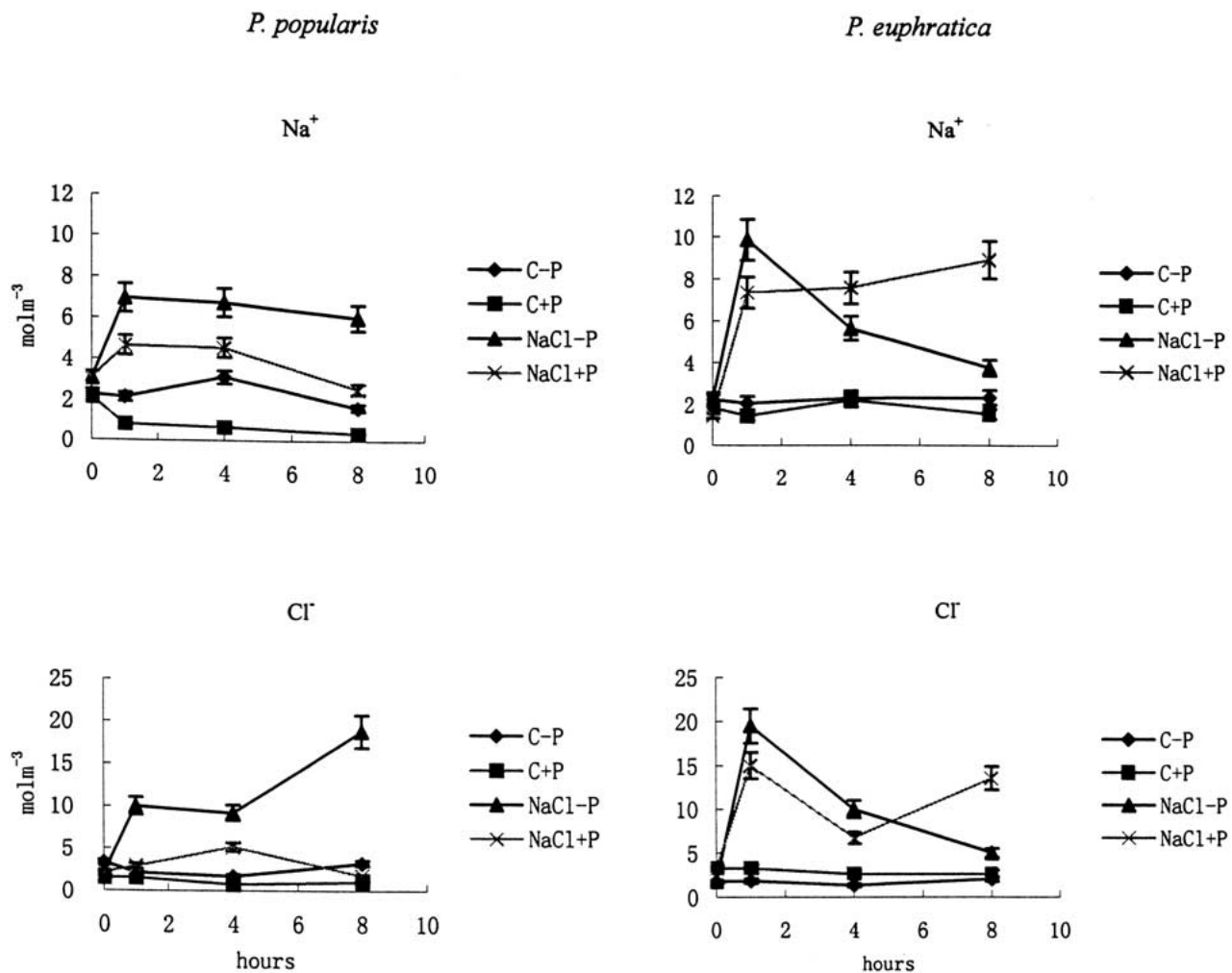
Figure 5. Effect of NaCl and pressurization (P) on root xylem Na^+ and Cl^- concentrations of *P. popularis* and *P. euphratica*. Each point is the mean of four plants and bars represent the standard error of the mean.

shown that ABA can be dragged by an apoplastic bypass flow directly across the endodermis into the xylem. Although pressure may enhance radial ABA transport, the higher and long-sustained ABA in the root xylem of pressurized *P. euphratica* consequently demonstrated that this genotype had a greater capacity to synthesize ABA under NaCl stress, which was partly due to the specific salt effects.

Osmotic Stress and Salt Effects on Gas Exchange

In general, the Pn response to an osmotic shock caused by PEG or NaCl followed the same trend as TRN in both genotypes, suggesting that the reduced Pn was the result of decreased stomatal conductance (Figures 1, 3). However, an inconsistent change was observed when pressure was applied to the root system of salinized *P. euphratica*, where water loss

was markedly enhanced but Pn was reduced (Figure 3). It appears that this discrepancy is associated with high levels of solutes in the xylem. Pressurized *P. euphratica* exported more Na^+ and Cl^- from the roots to the shoots under NaCl stress (Figure 5) causing a great buildup of salt in leaf apoplasts (data not shown), which may lead to physiological disturbances if the mesophyll cells cannot restrict their entry into the cytoplasm (Greenway and Munns 1980), for example, reduced activity of Rubisco (ribulose bisphosphate carboxylase-oxygenase), thus leading to decreased Pn. Moreover, ABA in the leaves of pressurized *P. euphratica* accumulated to a level that was even higher than nonpressurized plants (data not shown), resulting from root-drived ABA and pressure-reduced ABA recirculation from shoots to the roots. It is possible that the direct action of ABA on mesophyll photosynthesis contributed to the severely restricted Pn (Raschke and Hedrich 1985).

Genotypic Variation in Salinity Tolerance and the Relevance to ABA

Salt-tolerant *P. euphratica* maintained higher levels of ABA than the salt-sensitive genotype *P. populenis* under salt stress (Figures 2, 4), which is consistent with our previous report (Chen and others 2001) and other investigations conducted on rice (Moons and others 1995) and tomato (Dunlap and Binzel 1996). However, these results were contrary to other reports in which the levels of salt-induced ABA were negatively correlated with salinity tolerance (Asch and others 1995; He and Cramer 1996; Zhao and others 1991). These conflicting results indicate different mechanisms of salinity tolerance in different species. Our previous studies have shown that salt tolerance of *P. euphratica* was mainly attributed to its greater capacity to exclude salt from leaves, which was probably associated with the endogenous accumulation of ABA (Chen and others 2001). In the present study, results revealed that specific salt effects were presumably responsible for ABA accumulation, which is essential for *P. euphratica* to restrict root-to-shoot salt transport.

ABA limited Na^+ and Cl^- accumulation in the leaves (Karmoker and Van Steveninck 1979; Montero and others 1997), appearing to be the result of either restricted ion transport to the root xylem or stimulated vacuolar ion accumulation (Behl and Jeschke 1981), or both. Kasai and others (1993) found that ABA exerted a stimulatory effect on the H^+ transport activities of tonoplast-enriched membrane vesicles of barley roots, which may make a contribution to the compartmentalization of toxic ions (Na^+ and Cl^-) into the vacuoles (Bennett and Spanswick 1983; Blumwald and Poole 1985; Garbarino and Dupont 1988; Schumaker and Sze 1987). In accordance, X-ray microanalysis showed that root cortical vacuoles of *P. euphratica* functioned as storage areas for salt ions, especially Cl^- (Chen and others 2002; Fung and others 1996). More ions were sequestered in cortical vacuoles of *P. euphratica* when these ions were passing through the cortex to the xylem. As a result, ion loading into the xylem was lowered and subsequent axial transport was consequently limited (Chen and others 2002). Similarly, Jeschke (1984) found that a more salt-tolerant barley cultivar accumulated more Na^+ ions in vacuoles than a salt-sensitive cultivar, which lowered its flow to the shoot.

In this study, although Na^+ and Cl^- concentrations in root xylem of *P. euphratica* were higher than *P. populenis* after the initiation of NaCl stress, they both decreased to a lower level after 8 h of stress, whereas *P. populenis* stabilized Na^+ and Cl^- at a rel-atively higher and constant level during the period of stress (Figure 5). Accordingly, these results showed that *P. euphratica* had a greater capacity to restrict root-shoot salt transport. This may be associated with high levels of endogenous ABA which contributed to salt compartmentalization in vacuoles (Behl and Jeschke 1981).

Root xylem Na^+ and Cl^- in salinized *P. euphratica* reached a high level following the pressure treatment (Figure 5), suggesting that pressurization increased ion transport across the root into the xylem. Our previous study has shown that salinized *P. euphratica* accumulated salts in root cortical walls and compartmentalized Cl^- in cortical vacuoles (Chen and others 2002). Pressurizing of root systems consequently enhanced radial salt transport to the xylem vessels. A direct apoplastic pathway may contribute to the radial ion transport (Steudle and others 1993; Yeo and others 1987).

In summary, *P. euphratica* had a higher capacity to synthesize ABA under saline conditions as compared with the salt-sensitive genotype, *P. populenis*. We concluded that the long-sustained high levels of ABA in *P. euphratica* partially resulted from specific salt effects, in addition to an initial rapid rise of ABA that was caused mainly by osmotic stress. The higher capacity of *P. euphratica* in salt transport restriction, which contributes to its salt tolerance may be associated with the long-term ABA accumulation.

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REFERENCES

Asch F, Dorffling K, Dingkuhn M. 1995. Response of rice varieties to soil salinity and air humidity: A possible involvement of root-borne ABA. Plant Soil 177:11–19.

Behl R, Jeschke WD. 1981. Influence of abscisic acid on unidirectional fluxes and intracellular compartmentation of K^+ and Na^+ in excised barley root segments. Physiol Plant 53:95–100.

Bennett AB, Spanswick RM. 1983. Optical measurements of ΔpH and $\Delta \Psi$ in corn root membrane vesicles: Kinetic analysis of Cl^- effects on a proton translocating ATPase. J Membr Biol 71:95–107.

Blumwald E, Poole RJ. 1985. Nitrate storage and retrieval in *Beta vulgaris:* Effects of nitrate and chloride on proton gradients in tonoplast vesicles. Proc Natl Acad Sci USA 82:3683–3687.

Chazen O, Hartung W, Neumann PM. 1995. The different effects of PEG 6000 and NaCl on leaf development are associated with differential inhibition of root water transport. Plant Cell Environ 18:727–735.

Chen S, Wang S, Altman A, Hüttermann A. 1997. Genotypic variation in drought tolerance of poplar in relation to abscisic acid. Tree Physiol 17:797–803.

Chen S, Li J, Wang S, Hüttermann A, Altman A. 2001. Salt, nutrient uptake and transport, and ABA of *Populus euphratica*; a hybrid in response to increasing soil NaCl. Trees 15:186–194.

Chen S, Li J, Fritz E, Wang S, Hüttermann A. 2002. Sodium and chloride distribution in roots and transport in three poplar genotypes under increasing NaCl stress. Forest Ecology and Management 168:217–230.

Chen X, Wang S. 1992. Quantitative analysis of ABA, IAA and NAA in plant tissues by HPLC (in Chinese). Plant Physiol Comm 28:368–371.

Dunlap JR, Binzel ML. 1996. NaCl reduced indole-3-acetic acid levels in the roots of tomato plants independent of stress-induced abscisic acid. Plant Physiol 112:379–384.

Else MA, Davies WJ, Whitford PN, Hall KC, Jackson MB. 1994. Concentrations of abscisic acid and other solutes in xylem sap from root system of tomato and castor-oil plants are distorted by wounding and variable sap flow rates. J Exp Bot 45:317–323.

Fort C, Fauveau ML, Muller F, Label P, Granier A, Dreyer E. 1997. Stomatal conductance, growth and root signaling in young oak seedlings subjected to partial soil drying. Tree Physiol 17:281–289.

Freundl E, Steudle E, Hartung W. 1998. Water uptake by roots of maize and sunflower affects of abscisic acid and its concentration in the xylem. Planta 207:8–19.

Fung LE, Ma H, Wang S. 1996. X-ray microanalysis of ion distribution in salt tolerancy and salt intolerant poplar genotypes. J Beijing For Univ 5(2):23–30.

Garbarino J, Dupont FM. 1988. NaCl induces a Na^+/H^+ antiport in tonoplast vesicles from barley roots. Plant Physiol 86:231–236.

Gómez-Cadenas A, Tadeo FR, Primo-Millo E, Talon M. 1998. Involvement of abscisic acid and ethylene in the responses of citrus seedlings to salt shock. Physiol Plant 103:475–484.

Greenway H, Munns R. 1980. Mechanisms of salt tolerance in nonhalophytes. Ann Rev Plant Physiol 31:149–190.

He T, Cramer GR. 1996. Abscisic acid concentrations are correlated with leaf area reductions in two salt-stressed rapid-cycling *Brassica* species. Plant Soil 179:25–33.

Jeschke WD. 1984. K^+-Na^+ exchange at cellular membranes, intracellular compartmentation of cations, and salt tolerance. In: Staples RC, editor. Salinity tolerance in plants: Strategies for crop improvement. New York: John Wiley and Sons, pp 37–65.

Jeschke WD, Peuke AD, Pate JS, Hartung W. 1997. Transport, synthesis and catabolism of abscisic acid (ABA) in intact plants of castor bean (*Ricinus communis* L.) under phosphate deficiency and moderate salinity. J Exp Bot 48:1737–1747.

Karmoker JL, Van Steveninck RFM. 1979. The effect of abscisic acid on the uptake and distribution of ions in intact seedlings of *Phaseolus vulgaris* cv. Redland Pioneer. Physiol Plant 45:453–459.

Kasai M, Yamamoto Y, Maeshima M, Matsumoto H. 1993. Effects of in vivo treatment with abscisic acid and/or cytokinin on activities of vacuolar H^+ pumps of tonoplast-enriched membrane vesicles prepared from barley roots. Plant Cell Physiol 34:1107–1115.

Liang J, Zhang J. 1997. Collection of xylem sap at flow rate similar to in vivo transpiration flux. Plant Cell Physiol 38:1375–1381.

Mexal J, Fisher JT, Osteryoung J, Patrick Reid CP. 1975. Oxygen availability in polyethylene glycol solutions and its implications in plant-water relations. Plant Physiol 55:20–24.

Montero E, Cabot C, Barceló J, Poschenrieder C. 1997. Endogenous abscisic acid levels are linked to decreased growth of bush bean plants treated with NaCl. Physiol Plant 101:17–22.

Montero E, Cabot C, Poschenrieder CH, Barceló J. 1998. Relative importance of osmotic-stress and ion-specific effects on ABA-mediated inhibition of leaf expansion growth in *Phaseolus vulgaris*. Plant Cell Environ 21:54–62.

Moons A, Bauw G, Prinsen E, Van Montagu M, Van Der Straeten D. 1995. Molecular and physiological responses to abscisic acid and salts in roots of salt-sensitive and salt-tolerant Indica rice varieties. Plant Physiol 107:177–186.

Munns R. 1985. Na^+, K^+ and Cl^- in xylem sap flowing to shoots of NaCl-treated barley. J Exp Bot 36:1032–1042.

Passioura JB. 1980. The transport of water from soil to shoot in wheat seedlings. J Exp Bot 31:333–345.

Passioura JB, Munns R. 1984. Hydraulic resistance of plants. II. Effects of rooting medium and time of day in barley and lupin. Aust J Plant Physiol 11:341–350.

Plant AL, Cohen A, Moses MS, Bray EA. 1991. Nucleotide sequence and spatial expression pattern of a drought and abscisic acid-induced gene of tomato. Plant Physiol 97:900–906.

Raschke K, Hedrich R. 1985. Simultaneous and independent effects of abscisic acid on stomatal and the photosynthetic apparatus in whole leaves. Planta 163:105–118.

Ribaut JM, Pilet PE. 1991. Effects of water stress on growth, osmotic potential and abscisic acid content of maize roots. Physiol Plant 81:156–162.

Schumaker KS, Sze H. 1987. Decrease of pH gradients in tonoplast vesicles by NO_3^- and Cl^-: Evidence for H^+-coupled anion transport. Plant Physiol 83:490–496.

Schurr U, Schulze ED. 1995. The concentration of xylem sap constituents in root exudates, and in sap from intact, transpiring castor bean plants (*Ricinus communis* L). Plant Cell Environ 18:409–420.

Steudle E, Meshcheryakov AB. 1996. Hydraulic and osmotic properties of oak roots. J Exp Bot 47:387–401.

Steudle E, Murrmann M, Peterson CA. 1993. 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 103:335–349.

Thomas JC, McElwain EF, Bohnert HJ. 1992. Convergent induction of osmotic stress responses. Plant Physiol 100:416–423.

Wolf O, Jeschke WD, Hartung W. 1990. Long distance transport of abscisic acid in NaCl-treated plants of *Lupinus albus*. J Exp Bot 41:593–600.

Yeo AR, Yeo ME, Flowers TJ. 1987. The contribution of an apoplastic pathway to sodium uptake by rice roots in saline conditions. J Exp Bot 38:1141–1153.

Zhao K, Munns R, King RW. 1991. Abscisic acid synthesis in NaCl-treated barley, cotton and saltbush. Aust J Plant Physiol 18:17–24.