

Water absorption is affected by the nitrogen supply to rice plants

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Received: 19 March 2015 / Accepted: 10 July 2015 / Published online: 20 July 2015
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Abstract

Background and aims Plant growth and photosynthetic ability have been frequently demonstrated to increase with nitrogen (N) supply. N can also promote changes in root water absorption and shoot water status via mechanisms that remain poorly understood. This study aims at investigating the effects of N supply on water absorption.

Methods A hydroponic experiment with two independent rice varieties (cv. ‘Shangyou63’ hybrid *indica* and cv. ‘Yangdao6’ conventional *indica*, China) supplied by three distinct N levels was performed in a greenhouse. Physiological characteristics were analyzed after a few weeks.

Results Compared to low N supply ($20 \text{ mg}\cdot\text{L}^{-1}$), exposure to high N supply ($100 \text{ mg}\cdot\text{L}^{-1}$) increased the light-saturated photosynthetic rate (A) and water use efficiency (WUE) by 17 % and 22 %, respectively, in Shanyou63 and by 43 % and 26 %, respectively, in

Yangdao6. The leaf water potential was significantly decreased in Shanyou63 but not in Yangdao6. There were increases in the rate of water uptake and the root hydraulic conductance (L_r) under high N supply in both rice cultivars; these changes were accompanied by increased transcription levels of aquaporins (AQPs), decreased aerenchyma formation and root porosity, and decreased root lignin content. Under high N supply, Yangdao6 also exhibited much higher AQP activity, lower aerenchyma and root porosity compared with those of Shanyou63, indicating that Yangdao6 had an increased ability to absorb water compared with that of Shanyou63.

Conclusions The enhanced expression of AQPs and decreased root aerenchyma and lignin contributed to increased water absorption ability under high N supply. In addition, the responses of each of the two rice cultivars (hybrid and conventional) to N supply is related to their water uptake ability, resulting from root porosity and an increase in AQP activity.

Responsible Editor: Ad C. Borstlap.

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Keywords Water absorption · WUE · Leaf water potential · AQP · Aerenchyma

Introduction

Water and nitrogen are major limiting factors for plant growth and production (Evans 1983; Tezara et al. 1999; Lawlor 2002; Passioura 2007). Water and N uptake are generally associated with higher plants; for example, it has been demonstrated that nitrate uptake is positively

related to water uptake (Guo et al. 2002; Gorska et al. 2008a). Decreased hydraulic conductance as well as water deficits will reduce the leaf elongation rate, leaf potential, stomatal conductance (g_s), and nutrient uptake (Tanguilig et al. 1987; Saliendra et al. 1995).

Numerous studies have reported that water uptake into roots and transport to the shoot are often affected by root/rhizosphere water potential and oxygen deficiency, transpiration rate, and biotic or abiotic perturbation (Steudle 2000a; Maurel et al. 2008; Vandeleur et al. 2009). Root hydraulic conductance (L_r), an important parameter demonstrating water transport ability through root xylem, is plant species-dependent, which is mainly affected by the root morphology, anatomy and aquaporin (AQP) activity (Bramley et al. 2009). The formation of aerenchyma and the impermeable barrier to oxygen potential poses a large resistance to radial flow, which inevitably decreases the L_r (Colmer 2003). Inhibition of the root cortical cell hydraulic conductivity (L_c) could account for the decrease in L_r of wheat, while L_c may be related to AQP activity under the regulation of cytoplasmic pH, free Ca^{2+} , phosphorylation and H_2O_2 (Bramley et al. 2010; Bramley and Tyerman 2010). Vandeleur et al. (2005) reported that the water permeability of root plasma membrane vesicles was also strongly inhibited by reductions in the pH.

Low N can significantly reduce the effective leaf area as a result of reductions in the leaf size, chlorophyll content and CO_2 assimilation, leading to a subsequent decrease in the crop yield (Bennett et al. 1989; Dordas and Sioulas 2008). Conversely, increased N supply can improve the water use efficiency (WUE) and plant productivity (Lajtha and Whitford 1989; Green and Mitchell 1992; Livingston et al. 1999; Hobbie and Colpaert 2004). Many studies have focused on the effect of N on stomatal conductance or mesophyll conductance. For example, increasing the photosynthetic rate (A) following increased N investment in Rubisco with little or no effect on stomatal conductance (g_s) (Wang et al. 1998; Welander and Ottosson 2000) or with an increase in mesophyll conductance (g_m) (Warren and Adams 2006; Flexas et al. 2008) lead to an increase in WUE. Other work has also shown that A and g_s were directly proportional to the plant hydraulic conductance, suggesting that changes in the plant hydraulic conductance may affect the plant carbon gain (Hubbard et al. 2001). Whether N affected the plant hydraulic conductance by altering characteristics of the roots or leaves remains unclear,

although changes induced by N nutrition to each of these attributes can potentially affect L_r .

Previous studies have shown that low N can decrease the plant hydraulic conductance (Radin and Boyer 1982; Radin 1983). Low phosphorus (P) and low potassium (K) also showed effects that paralleled those of low N (Dordas and Sioulas 2008; Oddo et al. 2011). Plants grown on low P had a reduced leaf water potential, transpiration rate, and L_r , leading to reductions in the leaf area and shoot dry weight. The decrease in L_r was caused by a reduced radial conductivity, which could result from decreased root cell membrane permeability and from changes in apoplastic or symplastic pathways (Augé and Stodola 1990; Cui and Nobel 1992). Recent findings have suggested that the N availability potentially affected L_r via changes in AQP expression (Ishikawa-Sakurai et al. 2014). Another study reported that there was a rapid increase in L_r associated with the addition of NO_3^- , but similar effects were not observed for other anions, such as SO_4^{2-} and $H_2PO_3^-$. This response could be attributed to changes in root cell membrane hydraulic conductance by NO_3^- , which was related to a hydraulic signal and AQP activity but not to AQP expression or de novo protein synthesis (Gorska et al. 2008a, b). It is possible that the rapid increase in L_r in the presence of increased NO_3^- availability is an important trait that enhances the ability of plants to compete for the NO_3^- in soil (Gloser et al. 2007). Although these studies provided some explanation for the increase in L_r observed when the plant is supplied with N, how N uptake is affected by water uptake and the relationship between these two factors are still not completely understood.

Based on our previous cultivar selection experiment, we chose the two rice cultivars of ‘Shanyou63’ (hybrid *indica*, China) and ‘Yangdao6’ (conventional *indica*, China), which were widely planted in China and showed different responses of water uptake ability and photosynthesis to nitrogen forms and/or concentrations (Yang et al. 2012; Li et al. 2013). Our previous studies had shown that under well watered conditions, physiological effects of moderate N supply were independent of the applied N form (Yang et al. 2012). In consideration of the field environment, lowland rice roots are actually exposed to a mixed nitrate and ammonium supply in the rhizosphere (Briones et al. 2003; Kirk and Kronzucker 2005). In addition, aggravating water shortage, conventional flood irrigation methods should be replaced with intermittent irrigation or dry cultivation

in rice production. There will be an increase in nitrate content in these conditions. So, in the present study, we supplied even concentration of nitrate and ammonium, and assessed growth, leaf gas exchange, WUE, L_s , and factors affecting water absorption under various N supplies in two rice cultivars of Shanyou63 and Yangdao6. We discuss the effects of N on root anatomy and water transport pathways as well as the contribution of AQP to water uptake; we also comment on the differences between these two cultivars.

Materials and methods

Plant materials and growth conditions

Rice seeds (*Oryza sativa* L., cv. “Shanyou63” hybrid *indica* China, and “Yangdao6” conventional *indica* China) were sterilized in 10 % H_2O_2 for 30 min and transferred to moist filter paper for germination. When the seedlings developed an average of 2.5 visible leaves in distilled water, they were transplanted into 6-L rectangular containers ($30 \times 20 \times 10$ cm) with quarter-strength nutrient solution (for composition, see below) for 2 days. Then, the seedlings were transferred to half-strength nutrient solution for 3 days. Later, the seedlings were supplied with full-strength nutrient solution for 4 days. Next, the seedlings were supplied with low-N ($20 \text{ mg} \cdot \text{L}^{-1}$), moderate-N ($40 \text{ mg} \cdot \text{L}^{-1}$) and high-N ($100 \text{ mg} \cdot \text{L}^{-1}$) nutrient solutions. The nutrient solutions included the following components: macronutrients ($\text{mg} \cdot \text{L}^{-1}$): N as equal moles of $(\text{NH}_4)_2\text{SO}_4$ and $\text{Ca}(\text{NO}_3)_2$, 10 P as KH_2PO_4 , 40 K as K_2SO_4 and KH_2PO_4 , 40 Mg as $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 57 Ca as $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and micronutrients ($\text{mg} \cdot \text{L}^{-1}$): 2.0 Fe as Fe-EDTA ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), 0.5 Mn as $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.2 B as H_3BO_3 , 0.05 Mo as $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 0.01 Zn as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 Cu as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, and 2.8 Si as $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$. A nitrification inhibitor dicyandiamide (DCD, $1 \text{ mg} \cdot \text{L}^{-1}$) was added to each nutrient solution to prevent ammonium oxidation. The nutrient solutions were changed every 3 days, and the pH was adjusted to 5.50 ± 0.05 daily with 0.1 M HCl and 0.1 M NaOH. Each treatment included five replicates in a completely randomized design. The spatial arrangement of the treatments was randomized to avoid edge effects in the greenhouse.

Plants were grown in the greenhouse at a temperature of 25 ± 5 °C. The maximum light intensity at midday

was more than $1,500 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at the leaf level. Physiological and biochemical parameters were measured at tillering stage about 35–45 days after transplanting.

Biomass measurements

Plant samples were harvested and separated into root, stem, and leaf fractions at tillering stage. All samples were oven-dried at 105 °C for 30 min and then at 70 °C to a constant weight. The leaf area was photographed and then analyzed using Image-Pro Plus 6.0 software.

Gas exchange measurements

At tillering stage, the photosynthetic rate of the newly expanded leaves was measured using a Li-Cor 6400 portable photosynthesis open system with red-blue light from 09:00 to 11:00 AM. During the measurements, the leaf temperature was maintained at 28 °C, and the photosynthetic photo flux density (PPFD) was $1,500 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. In addition, the CO_2 concentration in the cuvette was adjusted to the atmospheric concentration of CO_2 ($390 \mu\text{mol} \cdot \text{mol}^{-1}$), and the relative humidity was maintained at 40 %. After equilibration to a steady state, the gas exchange parameters, including light-saturated photosynthetic rate (A), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and transpiration rate (T_r), were recorded. More details about measurement can be found in our previous studies or other studies (Long et al. 1996; Long and Bernacchi 2003; Li et al. 2009). Water use efficiency (WUE) was calculated as the ratio of A to T_r (A/T_r), and the stomata limitation (L_s) was calculated from the equation of $L_s = (A_r - A)/A_i$ (Farquhar and Sharkey 1982), where A_r is the light-saturated photosynthetic rate when C_i was $390 \mu\text{mol} \cdot \text{mol}^{-1}$.

Leaf water potential measurements

The leaf water potential was determined at 00:00–04:00 AM at tillering stage (Han et al. 2013). Newly expanded leaves were sampled, immediately followed by cutting into small pieces and spread evenly on the bottom of the sample cup to determine water potential using a WP4 Dewpoint Potential Meter (Decagon devices, Pullman, WA, USA).

Nitrogen absorption measurements

At tillering stage, each rice plant was transferred to a medium containing 20, 40 or 100 mg·L⁻¹ N as equal moles of NO₃⁻ and NH₄⁺ for 8 h. Nitrogen absorption was assessed by the amount of NO₃⁻ and NH₄⁺ remaining in the medium after the measurement period. The levels of NO₃⁻ and NH₄⁺ were determined using an Auto Analyzer 3 digital colorimeter (BRAN+LuEBBE). The reagents used for NO₃⁻ determination were 1 % sulfanilamide (C₆H₈N₂O₂S), 0.05 % N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD), 10 % (V/V) H₃PO₄, 4 % NaOH, 0.3 % (V/V) H₃PO₄, 0.001 % CuSO₄·5H₂O, 0.01 % ZnSO₄·7H₂O and 0.2 % hydrazine sulphate (N₂H₄·H₂SO₄). The reagents used for NH₄⁺ determination were 4 % tri-sodium citrate (C₆H₅Na₃O₇·2H₂O), 4 % sodium salicylate (Na₂C₇H₅O₃), 0.1 % sodium nitroprusside {Na₂[Fe(CN)₅NO]·2H₂O}, 4 % NaOH and 0.3 % dichloro-isocyanuric acid sodium salt dehydrate (DCI).

Water uptake rate and AQP activity measurements

The water uptake by intact plants with or without the addition of 0.1 mM HgCl₂ to hydroponic solutions was determined by the depletion of nutrient solution (by weight) between 10:30 to 11:30 AM (1 h) at tillering stage. The contribution of Hg-sensitive AQP to the water uptake was calculated from the difference in the depletion of nutrient solution between the control and HgCl₂-treated plants, and the water uptake of HgCl₂-treated plants was generally regarded as an apoplastic contribution. The water uptake per leaf area (LWU) was calculated from the ratio of total water uptake to total leaf area per plant.

Root hydraulic conductance measurements

Hydraulic conductance was measured using a high-pressure flow meter (HPFM, Decagon Devices) at 10:00–12:00 AM at tillering stage. The HPFM was attached to the detached root with an Omnifit connector. Positive pressure (P_i) was applied at a constant rate of 3–7 kpa·s⁻¹, allowing the slope of the relationship between flow (F) and pressure (P_i) to be obtained as a transient measurement (K). After measuring K , the root was oven-dried to a constant weight (W). The root hydraulic conductance (L_r ; kg·s⁻¹·g⁻¹·MPa⁻¹) was calculated as

the following: $L_r = K/W$; for method details, see Yang et al. (2012).

Root anatomy observations

At tillering stage, the root aerenchyma tissue was studied using fresh free-hand sections (Lux et al. 2005). The sections were cut from 6–7 cm segments above the root tip with a razor blade, transferred to a drop of water on a microscope slide and then observed using an Olympus BX51 light microscope. Images were recorded using a high-sensitivity CCD color camera system.

Lignin measurements

At tillering stage, the lignin content of the roots was estimated using the acetyl bromide procedure (Iiyama and Wallis 1990; Vander et al. 1998). The cell walls were prepared from frozen rice roots by grinding in liquid nitrogen, followed by three sequential extractions with 95 % ethanol, ethanol: n-hexane (1:2) twice and then distilled water. Lyophilized and vacuum oven-dried cell walls (20 mg) were transported into glass tubes with 2.5 ml acetyl bromide reagent [25 % (v/v) acetyl bromide in acetic acid] and then incubated in a 70 °C water bath for 30 min. Ice-cooled samples were then added to 2 ml of 2 M NaOH, 5 ml of acetic acid, and 0.2 ml of 7.5 M hydroxylamine hydrochloride, and the volume was adjusted with acetic acid to 20 ml. The absorbance of the resulting solution was measured using an ultraviolet (UV) spectrophotometer at 280 nm. The lignin content was calculated using the molar extinction coefficient of 17 L·g⁻¹·cm⁻¹ (Hatfield and Fukushima 2005).

Root porosity measurements

At tillering stage, the porosity of the roots was determined according to previous methods (Visser et al. 2000; Fan et al. 2003). Briefly, 0.8 g of fresh roots was cut into approximately 1–2 cm length segments and weighed (RFW, root fresh weight). A 25 ml Pyrex pycnometer flask filled with degassed water was weighed before (W_1) and after (W_2) the addition of these segments. Next, the root segments were transferred to a vial, which was also filled with degassed water and vacuum-infiltrated until no air bubbles appeared.

These vacuum-infiltrated root segments were retransferred to the above degassed water-filled pycnometer, and the pycnometer was weighed again (W_3). The root porosity was calculated as the following:

$$\text{Porosity}(\%) = [(W_3 - W_2) / (RFW + W_1 - W_2)] \times 100$$

RNA extraction and semi-quantitative RT-PCR

At tillering stage, tissues of the rice roots and newly expanded leaves were sampled and quickly frozen in liquid nitrogen at 10:00–11:00 AM, then ground in a pre-cooled mortar with a pestle. RNA was extracted from the frozen tissue powder with TRIZOL reagent (Invitrogen). For reverse transcription-polymerase chain reaction (RT-PCR), first-strand cDNA was synthesized using M-MLV reverse transcriptase (Promega, Madison, WI, USA). The PCR conditions used were 95 °C for 3 min followed by 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. The PCR products were subjected to agarose gel electrophoresis and stained with ethidium bromide. The signal intensity of the stained bands was imaged using a charge-coupled device (CCD) camera. All of the primers and cycle numbers used for PCR are listed in Table 1.

Statistical analysis of the data

Differences among treatments were analyzed with the SAS 9.0 statistical software package, using the analysis

Table 1 Sequences of primer pairs and cycles used for semi-quantitative PCR

Gene	Primer	Cycles
<i>OsPIP2;1</i>	F: 5'-CGAGATCATCGGCACCTTC-3' R: 5'-TGCTTCATCAACGCCTTCC-3'	28/30
<i>OsPIP2;2</i>	F: 5'-AGCGACGGCTACTCCAAG-3' R: 5'-ATCTATCCACGAGGCGAAAT-3'	28/30
<i>OsPIP2;3</i>	F: 5'-CGAAAGGCTCGGGATAAG-3' R: 5'-TCAGGACGTACTGGTGGT-3'	30/35
<i>OsPIP2;4</i>	F: 5'-AACGCCCGCGACTCATAT-3' R: 5'-GGAGAAAGAGCCCAACAAT-3'	26/26
<i>OsPIP2;5</i>	F: 5'-AAACGCAATGGGCAAAGA-3' R: 5'-GCGCCACGATGTAGAGGAT-3'	30/32
<i>OsPIP2;6</i>	F: 5'-TCATCGCCACGCTCCTGT-3' R: 5'-GGCATTGCGCTTTGGGTC-3'	30/32
<i>ACT</i>	F: 5'-GGAAGTGGTATGGTCAAGG-3' R: 5'-AGTCTCATGGATACCCGCAG-3'	26/26

of variance (ANOVA) program. Significant differences ($P < 0.05$) among treatments are indicated in the tables and figures by different letters.

Results

Effects of the various N supplies on plant growth

The shoot dry mass was increased with increasing N supply in both cultivars, while the root dry mass was decreased, resulting in a reduced root/shoot ratio under high N supply (Table 2). Compared with low N supply, the root/shoot ratios under high N supply were decreased by 56 % and 57 % in Shanyou63 and Yangdao6, respectively. The leaf area was increased significantly in Shanyou63, but there was no difference between moderate-N-supplied plants and high-N-supplied plants in Yangdao6.

Effects of the various N supplies on photosynthetic parameters

Light-saturated photosynthetic rate (A) was increased significantly under high N supply in both rice cultivars. Under low N and moderate N supplies, A was lower in Yangdao6 than in Shanyou63, while there was no significant difference between the two cultivars under high N supplies (Table 3). This finding indicated that, compared with the low N supply, the increase in A under high N supply was more pronounced in Yangdao6 than in Shanyou63.

Stomatal conductance (g_s) showed no significant difference under the three N supplies in Shanyou63, while it was increased in Yangdao6 supplied with high N (Table 3). The intercellular CO_2 concentration (C_i) was decreased from 319 to 303 $\mu\text{mol mol}^{-1}$ in Shanyou63 and was approximately 310 $\mu\text{mol mol}^{-1}$ under all three N supplies in Yangdao6. The leaf transpiration rate (T_r) showed no significant differences in Shanyou63, while an increase was observed in Yangdao6. A comparison of the two cultivars revealed that the T_r was lower in Yangdao6 than in Shanyou63, particularly under low N and moderate N supplies. WUE was increased with increasing N supplies in both cultivars, and this effect was comparable under all three of the N supplies between both cultivars (Table 3 and Fig. 1). Yangdao6 had lower stomatal limitation (L_s) than that of Shanyou63 under high N supply.

Table 2 Effects of various N supplies on the plant dry weight (g·plant⁻¹), root/shoot ratio, leaf area (cm²·plant⁻¹) and the number of tillers (No.·plant⁻¹) in rice seedlings

Treatments		Root g·plant ⁻¹	Stem g·plant ⁻¹	Leaf g·plant ⁻¹	Root/Shoot	Leaf area cm ² ·plant ⁻¹
Shanyou63	Low-N	0.62±0.09a	0.80±0.05c	0.76±0.08c	0.40±0.03a	309±32c
	Moderate-N	0.53±0.06ab	1.02±0.10b	1.28±0.12b	0.23±0.01b	469±44b
	High-N	0.52±0.03b	1.29±0.06a	1.70±0.09a	0.17±0.01c	618±31a
Yangdao6	Low-N	0.54±0.01ab	0.66±0.03d	0.66±0.05c	0.41±0.02a	248±18d
	Moderate-N	0.46±0.06b	0.86±0.12c	1.15±0.08b	0.23±0.01b	420±30b
	High-N	0.35±0.02c	0.80±0.05c	1.21±0.07b	0.18±0.00c	423±7b

Rice plants were supplied with three levels of N supply (Low-N, 20 mg·L⁻¹; Moderate-N, 40 mg·L⁻¹; High-N, 100 mg·L⁻¹). Data are given as the mean ± SD of five replications. Significant differences ($P < 0.05$) among treatments are indicated by different letters

Effects of the various N supplies on plant water status and related parameters

The root hydraulic conductance (L_r) was also increased under high N supply in both rice cultivars (Fig. 2). Compared with the low N supply, a 168 % increase was observed in Shanyou63 and a 324 % increase was observed in Yangdao6 under high N supplies. There was no difference in the low-N-supplied plants between the two cultivars, but higher levels of L_r were observed in Yangdao6 compared with Shanyou63 under both moderate and high N supplies.

The leaf water potential (ψ_{leaf}) was decreased significantly in Shanyou63 supplied with high N, but no significant difference was observed in Yangdao6. Under low N supply, ψ_{leaf} was similar between the two cultivars, but it was lower in Shanyou63 than in Yangdao6 under both moderate and high N supplies (Fig. 3).

The root water uptake rate was increased with increases in the N supply in both rice cultivars (Table 4). Compared with low N supply, this parameter was increased by 292 % and 255 %, respectively, in Shanyou63 and Yangdao6 under high N supply. Under low or moderate N supply, the root water uptake rate was similar in both cultivars, while under high N supply, it was higher in Yangdao6 than in Shanyou63.

The addition of 0.1 mM HgCl₂ significantly suppressed the root water uptake rate in both cultivars, but it also increased with an increased N supply. Under high N supply, Yangdao6 had an increased water uptake rate than that of Shanyou63 in response to HgCl₂ treatment. When the HgCl₂ percentage of inhibition was calculated, it was shown that the magnitude of the decrease was greater and this effect was more pronounced in Yangdao6 than in Shanyou63 (Table 4). Compared with moderate N supply, there was a significant increase in the water uptake per leaf area (LWU) under high N supply in Yangdao6, but not in Shanyou63 (Table 4).

Table 3 Effects of various N supplies on photosynthetic parameters and WUE in rice seedlings. Net photosynthetic rate (A , $\mu\text{molCO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), stomatal conductance (g_s , $\text{molCO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$),intercellular CO₂ concentration (C_i , $\mu\text{mol}\cdot\text{mol}^{-1}$), transpiration rate (T_r , $\text{mmolH}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), water use efficiency (WUE, $\mu\text{molCO}_2\cdot\text{mmol}^{-1}\text{H}_2\text{O}$), and stomata limitation (L_s)

Treatments		A $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$	g_s $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$	C_i $\mu\text{mol}\cdot\text{mol}^{-1}$	T_r $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$	WUE $\mu\text{mol}\cdot\text{mmol}^{-1}$	L_s
Shanyou63	Low-N	20.34±0.91c	0.38±0.03a	319±10a	11.73±0.15a	1.72±0.08c	0.26±0.03bc
	Moderate-N	23.34±0.11a	0.41±0.01a	311±7ab	11.54±0.20ab	2.02±0.04b	0.26±0.00bc
	High-N	23.83±1.03a	0.39±0.02a	303±9b	11.34±0.46ab	2.10±0.11ab	0.27±0.03b
Yangdao6	Low-N	16.83±0.96d	0.24±0.04c	311±11ab	9.59±0.72d	1.76±0.22c	0.34±0.04a
	Moderate-N	22.39±0.68b	0.31±0.03b	307±10b	10.67±0.95c	2.10±0.07ab	0.25±0.02bc
	High-N	24.07±0.64a	0.39±0.04a	306±8b	10.91±0.15bc	2.22±0.07a	0.23±0.02c

Plant growth condition and data representation are the same as shown in Table 2

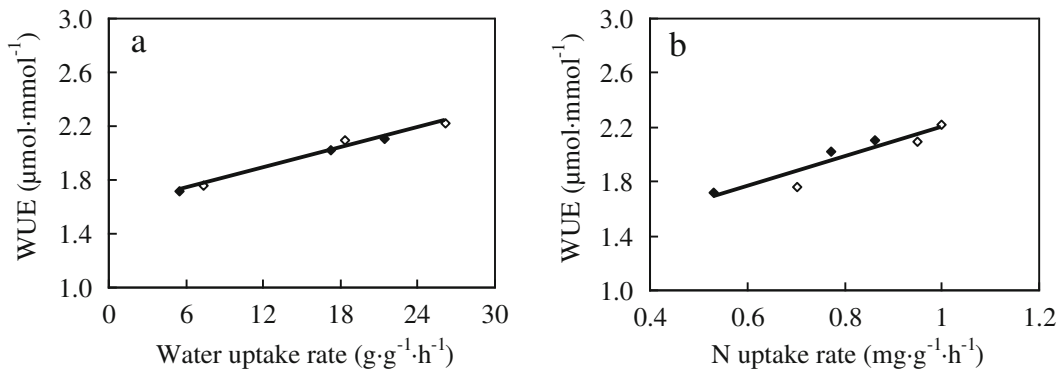


Fig. 1 The relationship between WUE and the water uptake rate (a), WUE and the N uptake rate (b). Filled squares and open squares represent Shanyou63 and Yangdao6, respectively. The

lines represent linear regression, and the regression equations are (a) $y=0.0249x+1.5876$, $R^2=0.9817$, $P=0.000$; (b) $y=1.0995x+1.1019$, $R^2=0.8789$, $P=0.006$

Effects of the various N supplies on root aerenchyma, lignin and porosity

To study the effects of root anatomy and morphology on the water uptake, the aerenchyma tissue was observed, and the root porosity and lignin content were also measured. The transverse freehand sections from the root showed evidence of aerenchyma and fine intercellular spaces (Fig. 4). High N visibly decreased the formation of aerenchyma in both rice cultivars. In addition, Yangdao6 had less aerenchyma than did Shanyou63 under high N supply.

Root porosity was also significantly decreased in both cultivars, but there was no difference between moderate and high N supplies in Shanyou63 (Fig. 5). Compared with a low N supply, root porosity was

decreased by 19 % and 56 % in Shanyou63 and Yangdao6, respectively, under high N supply.

The lignin content of the roots exhibited a similar tendency to that of the root porosity. Compared with low N supply, the former parameter was decreased by 24 % and 26 % under high N supply in Shanyou63 and Yangdao6, respectively. There was no difference in the lignin content between two cultivars at each N supply (Fig. 6).

Effects of the various N supplies on the expression of the leaf and root AQP genes

The mRNA levels of the plasma membrane intrinsic proteins (PIP2s) genes were analyzed by reverse transcription-PCR (RT-PCR) in the leaves and roots. Three replicates rendered qualitatively similar results.

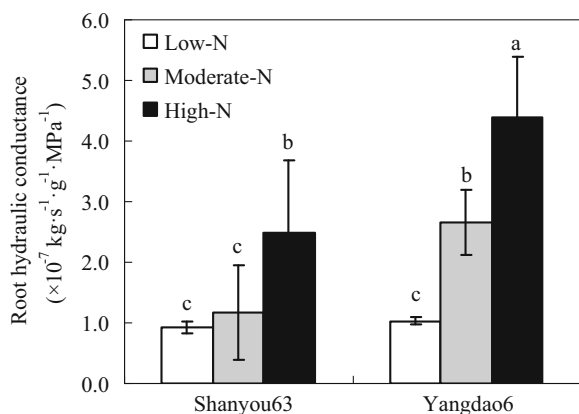


Fig. 2 Effects of various N supplies on the root hydraulic conductance of rice seedlings. Plant growth conditions are the same as those shown in Table 2. Data are represented as the mean of five replicates, and the bars indicate the SD. Significant differences ($P<0.05$) are indicated by different letters

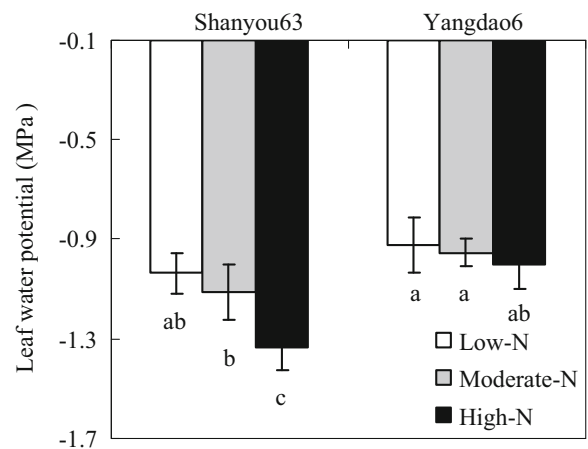


Fig. 3 Effects of various N supplies on the leaf water potential of newly expanded leaves in rice seedlings. Plant growth conditions and data representations are the same as those shown in Fig. 2

Table 4 Effects of various N supplies on the water uptake rate (with or without HgCl_2) based on per unit root dry weight for 1 h [$\text{gH}_2\text{O}\cdot(\text{g root DW})^{-1}\cdot\text{h}^{-1}$] and the decrease in response to the addition of 0.1 mM HgCl_2 to rice plants. The total water uptake

per plant normalized to leaf area (LWU) under control condition was also calculated as the ratio of total water uptake to total leaf area [$\text{gH}_2\text{O}\cdot(\text{m Leaf})^{-2}\cdot\text{s}^{-1}$]

Treatments		Control $\text{g}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$	HgCl_2 $\text{g}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$	Decreased percentage %	LWU $\text{g}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$
Shanyou63	Low-N	5.45±0.42d	5.10±0.08d	6.35	1.82±0.14e
	Moderate-N	17.21±1.43c	11.77±0.95c	31.65	3.24±0.17bc
	High-N	21.38±1.28b	14.32±0.86b	33.02	3.00±0.18c
Yangdao6	Low-N	7.37±1.20d	6.17±0.22d	16.34	2.64±0.29d
	Moderate-N	18.33±0.42c	11.85±0.27c	35.35	3.32±0.02b
	High-N	26.20±1.39a	15.75±1.43a	39.87	3.64±0.16a

Plant growth condition and data representation are the same as shown in Table 2. The LWU was calculated under control condition (without HgCl_2)

Representative examples are shown in Figs. 7 and 8. The expression levels of *OsPIP2;4* increased with increasing N supplies in the leaves of both rice cultivars. The leaf mRNA levels of *OsPIP2;1*, *OsPIP2;2* and *OsPIP2;3* were increased in Yangdao6, while there was no difference in Shanyou63. The root mRNA levels of *OsPIP2;1* and particularly *OsPIP2;4*, *OsPIP2;5* were significantly increased with increasing N supplies in both rice cultivars. The other PIPs expression in roots was nearly the same among the various N supplies.

Discussion

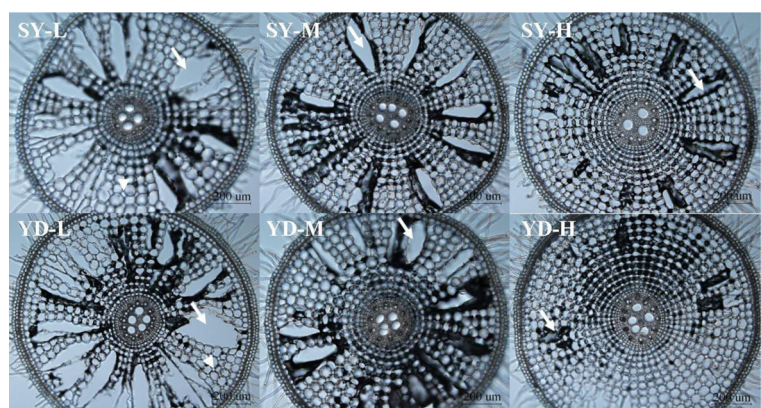
Water absorption, photosynthetic rate and WUE are increased under high N supply

Plant growth or crop productivity is always correlated with the N supply, and high N supply can improve

photosynthesis and the shoot dry weight but inhibit root growth, resulting in a decreased root/shoot ratio (Linkohr et al. 2002; Tian et al. 2005; Li et al. 2009). In the present study, we observed similar patterns in both rice cultivars, with an increased shoot weight and lower root/shoot ratio under high N supply (Table 2). Analysis of the gas exchange parameters illustrated that the photosynthetic rate (*A*) was increased with increasing N supply in both rice cultivars, but the magnitude of the increase varied. Compared with low N supply, *A* was increased by 17 % and 43 % in Shanyou63 and Yangdao6, respectively, under high N supply. This finding may have been caused by a lower stomatal limitation (L_s) in Yangdao6 compared with Shanyou63 under high N supply (Table 3).

Compared with low N treatment, the instantaneous WUE was increased by 22 % and 26 % under high N supply in Shanyou63 and Yangdao6, respectively; these findings are consistent with those obtained in previous

Fig. 4 Observation of aerenchyma tissue (arrows) and fine intercellular spaces (arrowheads) in rice root by free-hand section using bright field microscopy. SY and YD represent Shanyou63, Yangdao6; L, M and H represent low-N, moderate-N and high-N, respectively. Scale bars=200 μm



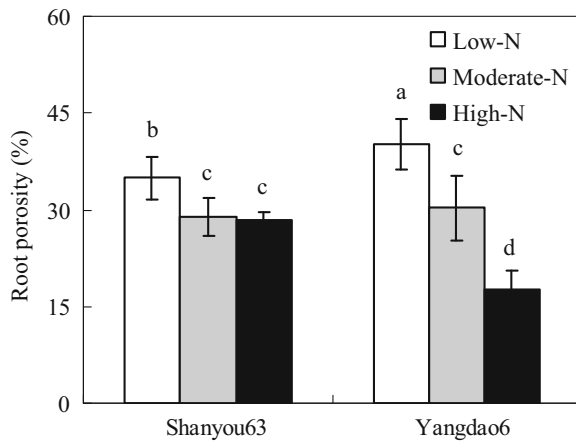


Fig. 5 Effects of various N supplies on the root porosity of rice seedlings. Plant growth conditions and data representations are the same as those shown in Fig. 2

studies (Guehl et al. 1995; Livingston et al. 1999; Hobbie and Colpaert 2004). Livingston et al. (1999) indicated that the higher WUE of white spruce with increased N supply was due to a decrease in g_s . In our study, there was an increase in A , while g_s was constant or slightly increased with increasing N supply, particularly in Yangdao6 (Table 3). Thus, we suggest that g_s was not responsible for the higher WUE in rice. We found a strong positive correlation not only between WUE and the N uptake rate but also between WUE and the water uptake rate (Fig. 1), indicating that the N uptake or water uptake might be a crucial factor influencing changes in WUE.

It is well known that the roots are the most important factor in water uptake for plant water relations. The root

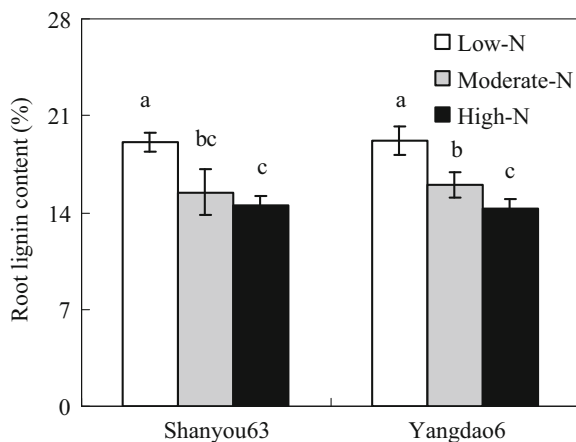


Fig. 6 Effects of various N supplies on the root lignin content of rice seedlings. Plant growth conditions and data representations are the same as those shown in Fig. 2

hydraulic conductivity (L_r) reflects the ability of the root to absorb water and may vary in response to external or internal factors, such as the water status due to drought or salinity or the demand for water from the shoot due to transpiration, the nutritional state, changes in the anatomy and structure of the root and AQP activity (Steudle 2000a). The decreased L_r temporarily decreased the xylem hydrostatic potential and consequently the leaf water potential (Else et al. 2001), which significantly affected plant water relations.

The present study showed that with an increasing N supply, L_r was increased (Fig. 2), which was accompanied by increased water absorption (Table 4). There was a significant positive correlation ($R^2=0.75$) between water absorption and the hydraulic conductance. The leaf water potential was also affected by the various N supplies, while each of the two cultivars showed a distinct response (Fig. 3). Yangdao6 had an increased leaf water potential compared with that of Shanyou63 under high N supply; these results suggested that Yangdao6 had a more favourable water status due to the higher L_r .

Root anatomy, morphology and AQPs are responsible for the ability to absorb water

In the root cylinder, there are three parallel pathways for water movement, i.e., the apoplastic, symplastic and transcellular pathways, with the latter two representing cell-to-cell pathways (Steudle 2000b). The apoplastic pathway resistance is strongly associated with the formation of the aerenchyma (Yang et al. 2012) and suberized and/or lignified cell walls (North and Nobel 1995; Hose et al. 2001). Many studies have indicated that N-, P- or sulfur (S)-deficiency induces aerenchyma formation in the root (Drew et al. 1989; Bouranis et al. 2003; Fan et al. 2007; Postma and Lynch 2011) and an increase in the lignin and/or suberin of the root or stem (Entry et al. 1998; Fritz et al. 2006; Pitre et al. 2007). The formed aerenchyma and lignin will inevitably inhibit L_r and water uptake via the apoplastic pathway. Taken together, our results illustrated that with an increasing N supply, aerenchyma formation, porosity and lignin were significantly decreased in the root (Figs. 4, 5 and 6), thereby improving the apoplastic water uptake (Table 4). Both porosity and lignin showed significant negative correlations with the water uptake rate, which was consistent with the results of previous studies (Yang et al. 2012). Under high N supply, the aerenchyma and

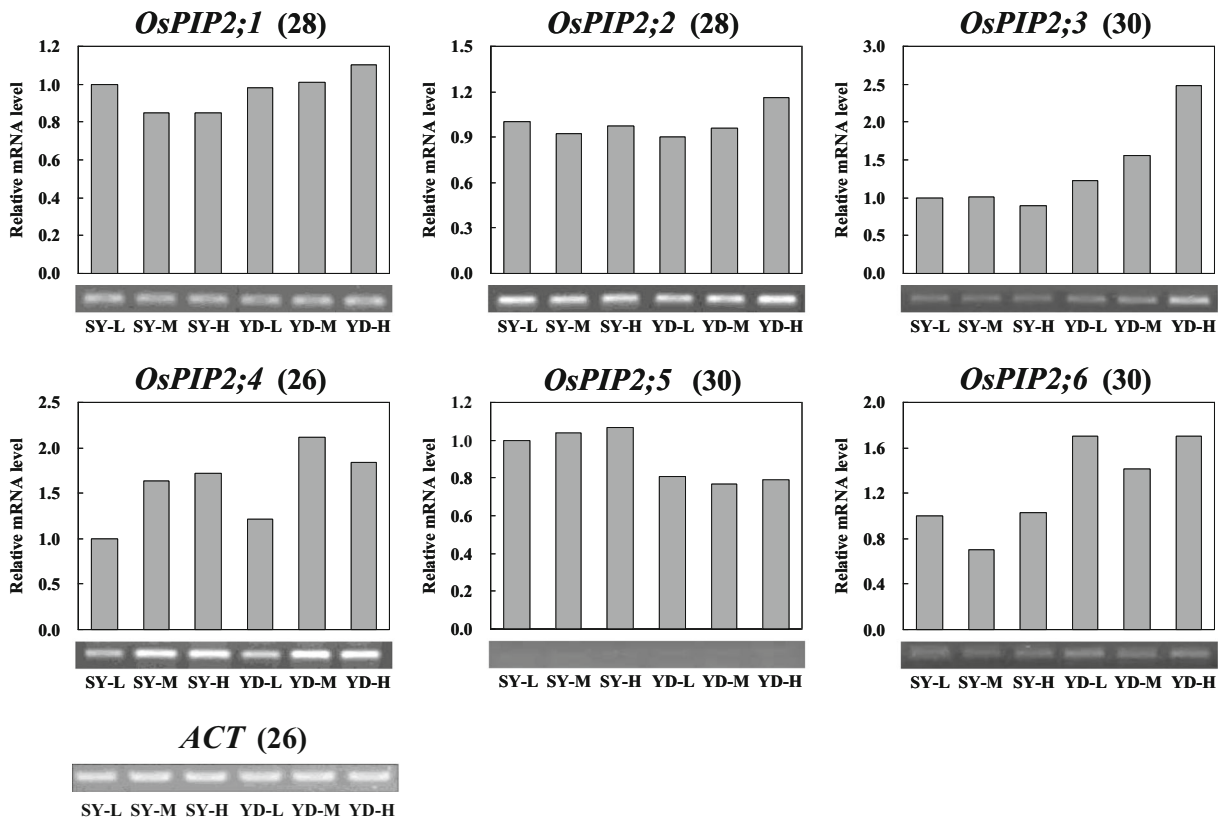


Fig. 7 Effects of various N supplies on leaf AQP expression in rice seedlings. Total RNA fractions were prepared from the roots and newly expanded leaves as described in the Materials and Methods section. The mRNA levels were analyzed by semi-quantitative RT-PCR using specific primers as shown in Table 1. The PCR products were separated on agarose gel and imaged after

ethidium bromide staining. Photographic images are shown at the bottom of each graph. The numbers in parentheses indicate the number of PCR cycles. SY and YD represent Shanyou63, Yangdao6; L, M and H represent low-N, moderate-N and high-N, respectively. The *OsActin* (*ACT*) gene was used for an internal standard

root porosity of Yangdao6 were significantly lower than those of Shanyou63, which might underlie the increased ability to absorb water in Yangdao6.

In the cell-to-cell pathway, the water molecules need to diffuse through the plasma membrane into the cytoplasm. During this process, the AQPs embedded in the membrane play an important role in water transport (Steudle and Peterson 1998; Steudle 2001; Kaldenhoff and Fischer 2006). Under well-watered conditions, the contribution of AQP to L_r was approximately 35 %–75 % (Maggio and Joly 1995; Lu and Neumann 1999; Wan and Zwiazek 1999; Barrowclough et al. 2000; North and Nobel 2000; Martre et al. 2001). Hacke et al. (2010) showed that seven AQPs were upregulated in the stem xylem of hybrid poplar under high N conditions. It is also possible that N or P deficiency may have decreased the activity and/or content of AQPs, further reducing the L_r (Carvajal et al. 1996; Clarkson et al.

2000). In the present study, Hg-sensitive AQP activity was determined by the discrepancy in the water uptake rate with and without the addition of 0.1 mM HgCl_2 , an AQP inhibitor (Javot and Maurel 2002; Wan et al. 2004; Beaudette et al. 2007). This finding showed that independent of the N supply and compared with the control (without HgCl_2), the water uptake rates in all HgCl_2 treatments decreased in both rice cultivars (Table 4). The decreased percentage represents the Hg-sensitive AQP activity, which was higher under high N supply, demonstrating that the high-N-supplied plants had an increased ability to take up water via the cell-to-cell pathway. We also investigated the transcription levels of *OsPIPs* that is a subfamily of AQPs using RT-PCR. The root mRNA expression levels of *OsPIP2;1*, *OsPIP2;4* and *OsPIP2;5* were significantly increased with increasing N concentration in both rice cultivars, which may also result in an increased ability to take up

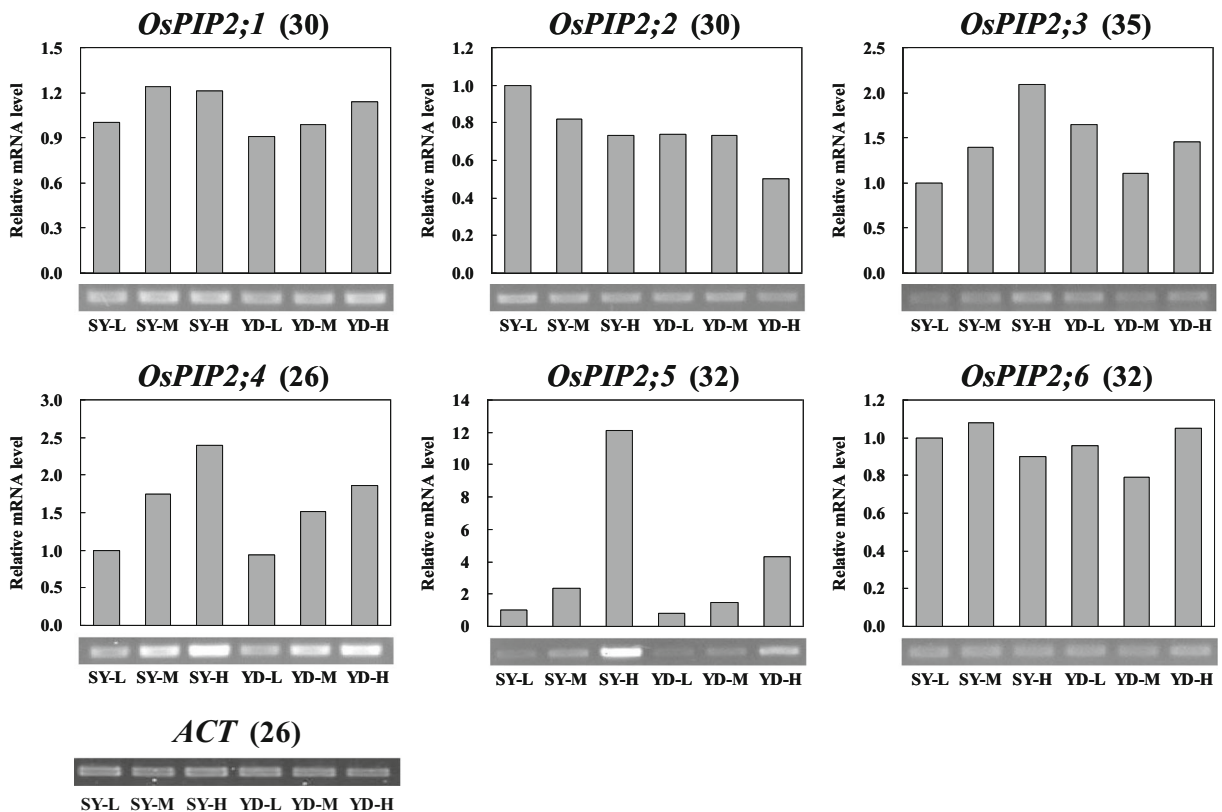


Fig. 8 Effects of various N supplies on root AQP expression in rice seedlings. The procedure and data representations are the same as those shown in Fig. 7

water via the cell-to-cell pathway of high-N plants. Upon comparison of the two rice cultivars, the mRNA expression levels of Shanyou63 were similar or slightly higher than those of Yangdao6, but the contribution of Hg-sensitive AQPs to water uptake was higher in Yangdao6 than in Shanyou63 regardless of the N supply (Table 4); these findings suggest that the root AQP activity plays a more important role in water uptake in Yangdao6 than in Shanyou63, which might account for the increased ability to take up water in Yangdao6 than in Shanyou63 under high N supply.

Differential response of water absorption under the various N supplies among the rice cultivars

Although the ability to take up water and the L_r both increased with the N supply, they were significantly higher in Yangdao6 than in Shanyou63 under high N supply, and the WUE was lower in Shanyou63 (Table 3). As previously described, this effect was caused by differences in the aerenchyma, root porosity

and root AQP activity between Yangdao6 and Shanyou63. In addition, although the root/shoot ratio decreased with the N supply, and proportion of the decrease was consistent from Shanyou63 to Yangdao6 (Table 2), the transpiration rate (T_r) in Shanyou63 was higher than that in Yangdao6, suggesting that Shanyou63 had a much higher water loss than that of Yangdao6. In addition, Shanyou63 had a reduced ability to absorb water than that of Yangdao6 under high N supply. When we calculated the total water uptake per leaf area, the results also showed the significant lower values in Shanyou63 (Table 4). This indicates relative water absorption ability on the base of leaf area under high N supply was lower in Shanyou63 compared with Yangdao6. Some studies reported that the decrease in leaf water potential was correlated with the changes in structure of cell or tissue of plants (Morgan 1986; Shangguan et al. 2000) or osmolytes, such as proline (Nayyar 2003). The changed leaf structure and/or osmolytes of Shanyou63 under high N condition might be the potential candidate for the decreased leaf water

potential. Moreover, the mRNA expression levels in the leaves of Yangdao6 were higher than those of Shanyou63, particularly *OsPIP2;3* and *OsPIP2;6* under high N supply. Parent et al. (2009) showed that water-deprived maize plants with high AQP expression and activity had an increased rate of recovery of leaf water potential after re-watering. Higher AQP expression in the leaf may be used for fine water regulation, which decreases water loss and increases WUE, thereby contributing to the maintenance of a favorable plant water status. In this study we provided even concentrations of nitrate and ammonium, we do not explore which N form may dominate the physiological effects observed here. But, in our previous studies there were no significant differences in L_s , root xylem sap flow rate, aerenchyma formation and root porosity between the nitrate fed plants and ammonium fed plants under non-water stress condition (Yang et al. 2012). The differences in nitrogen form effects on water relations were mainly observed under water stress condition. By varying nitrate and ammonium concentrations, the contribution of the different N forms to the physiological effects will be explored in a future study.

It can be concluded that under high N supply, decreased aerenchyma, porosity and lignin content of the roots, as well as increased AQP expression, were responsible for the high water absorption rate. Compared with the characteristics of Shanyou63, the greatly reduced aerenchyma and porosity of the root, higher hydraulic conductivity, and higher AQP activity in Yangdao6 resulted in increased WUE and water potential. These findings indicated that increasing N fertilization might increase the water absorption, WUE and production of rice plants via changes in the root properties.

Acknowledgments This work was supported by the National Basic Research Program of China (2013CB127403), the National Natural Science Foundation of China (31172020 and 31272236), Jiangsu Postdoctoral Science Foundation (1402148C) and China Postdoctoral Science Foundation (2015M571768).

References

- Augé R, Stodola A (1990) An apparent increase in symplastic water contributes to greater turgor in mycorrhizal roots of droughted rose plants. *HortSci* 25:1096–1096
- Barrowclough DE, Peterson CA, Steudle E (2000) Radial hydraulic conductivity along developing onion roots. *J Exp Bot* 51:547–557
- Beaudette PC, Chlup M, Yee J, Emery RJN (2007) Relationships of root conductivity and aquaporin gene expression in *Pisum sativum*: diurnal patterns and the response to $HgCl_2$ and ABA. *J Exp Bot* 58:1291–1300
- Bennett JM, Mutti LSM, Rao PSC, Jones JW (1989) Interactive effects of nitrogen and water stresses on biomass accumulation, nitrogen uptake, and seed yield of maize. *Field Crop Res* 19:297–311
- Bouranis DL, Chorianopoulou SN, Siyiannis VF, Protonotarios VE, Hawkesford MJ (2003) Aerenchyma formation in roots of maize during sulphate starvation. *Planta* 217:382–391
- Bramley H, Tyerman SD (2010) Root water transport under waterlogged conditions and the roles of aquaporins. In: Mancuso S, Shabala S (eds) *Waterlogging signalling and tolerance in plants*. Springer, Berlin, pp 151–180
- Bramley H, Turner NC, Turner DW, Tyerman SD (2009) Roles of morphology, anatomy, and aquaporins in determining contrasting hydraulic behavior of roots. *Plant Physiol* 150:348–364
- Bramley H, Turner NC, Turner DW, Tyerman SD (2010) The contrasting influence of short-term hypoxia on the hydraulic properties of cells and roots of wheat and lupin. *Funct Plant Biol* 37:183–193
- Briones AM Jr, Okabe S, Umehiya Y, Ramsing NB, Reichardt W, Okuyama H (2003) Ammonia-oxidizing bacteria on root biofilms and their possible contribution to N use efficiency of different rice cultivars. *Plant Soil* 250:335–348
- Carvajal M, Cooke D, Clarkson D (1996) Responses of wheat plants to nutrient deprivation may involve the regulation of water-channel function. *Planta* 199:372–381
- Clarkson DT, Carvajal M, Henzler T, Waterhouse RN, Smyth AJ, Cooke DT, Steudle E (2000) Root hydraulic conductance: diurnal aquaporin expression and the effects of nutrient stress. *J Exp Bot* 51:61–70
- Colmer T (2003) Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. *Plant Cell Environ* 26:17–36
- Cui M, Nobel PS (1992) Nutrient status, water uptake and gas exchange for three desert succulents infected with mycorrhizal fungi. *New Phytol* 122:643–649
- Dordas CA, Sioulas C (2008) Safflower yield, chlorophyll content, photosynthesis, and water use efficiency response to nitrogen fertilization under rainfed conditions. *Ind Crop Prod* 27:75–85
- Drew MC, He CJ, Morgan PW (1989) Decreased ethylene biosynthesis, and induction of aerenchyma, by nitrogen- or phosphate-starvation in adventitious roots of *Zea mays* L. *Plant Physiol* 91:266–271
- Else MA, Coupland D, Dutton L, Jackson MB (2001) Decreased root hydraulic conductivity reduces leaf water potential, initiates stomatal closure and slows leaf expansion in flooded plants of castor oil (*Ricinus communis*) despite diminished delivery of ABA from the roots to shoots in xylem sap. *Physiol Plantarum* 111:46–54
- Entry JA, Runion GB, Prior SA, Mitchell RJ, Rogers HH (1998) Influence of CO_2 enrichment and nitrogen fertilization on tissue chemistry and carbon allocation in longleaf pine seedlings. In: Box J (ed) *Root demographics and their efficiencies in sustainable agriculture, grasslands and forest ecosystems*. Springer, Netherlands, pp 3–18
- Evans JR (1983) Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L.). *Plant Physiol* 72:297–302

- Fan M, Zhu J, Richards C, Brown KM, Lynch JP (2003) Physiological roles for aerenchyma in phosphorus-stressed roots. *Funct Plant Biol* 30:493–506
- Fan M, Bai R, Zhao X, Zhang J (2007) Aerenchyma formed under phosphorus deficiency contributes to the reduced root hydraulic conductivity in maize roots. *J Integr Plant Biol* 49:598–604
- Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. *Ann Rev Plant Physiol* 33:317–345
- Flexas J, Ribas-Carbó M, Diaz-Espejo A, Galmes J, Medrano H (2008) Mesophyll conductance to CO₂: current knowledge and future prospects. *Plant Cell Environ* 31:602–621
- Fritz C, Palacios-Rojas N, Feil R, Stitt M (2006) Regulation of secondary metabolism by the carbon-nitrogen status in tobacco: nitrate inhibits large sectors of phenylpropanoid metabolism. *Plant J* 46:533–548
- Gloser V, Zwieniecki MA, Orians CM, Holbrook NM (2007) Dynamic changes in root hydraulic properties in response to nitrate availability. *J Exp Bot* 58:2409–2415
- Gorska A, Ye Q, Holbrook NM, Zwieniecki MA (2008a) Nitrate control of root hydraulic properties in plants: translating local information to whole plant response. *Plant Physiol* 148:1159–1167
- Gorska A, Zwieniecka A, Holbrook NM, Zwieniecki MA (2008b) Nitrate induction of root hydraulic conductivity in maize is not correlated with aquaporin expression. *Planta* 228:989–998
- Green T, Mitchell R (1992) Effects of nitrogen on the response of loblolly pine to water stress I. Photosynthesis and stomatal conductance. *New Phytol* 122:627–633
- Guehl J, Fort C, Ferhi A (1995) Differential response of leaf conductance, carbon isotope discrimination and water-use efficiency to nitrogen deficiency in maritime pine and pedunculate oak plants. *New Phytol* 131:149–157
- Guo S, Brück H, Sattelmacher B (2002) Effects of supplied nitrogen form on growth and water uptake of French bean (*Phaseolus vulgaris* L.) plants. *Plant Soil* 239:267–275
- Hacke UG, Plavcová L, Almeida-Rodriguez A, King-Jones S, Zhou W, Cooke JEK (2010) Influence of nitrogen fertilization on xylem traits and aquaporin expression in stems of hybrid poplar. *Tree Physiol* 30:1016–1025
- Han Y, Wang Y, Jiang H, Wang M, Korpelainen H, Li C (2013) Reciprocal grafting separates the roles of the root and shoot in sex-related drought responses in *Populus cathayana* males and females. *Plant Cell Environ* 36:356–364
- Hatfield R, Fukushima RS (2005) Can lignin be accurately measured? This paper was originally presented at the Lignin and Forage Digestibility Symposium, 2003 CSSA Annual Meeting, Denver, CO. *Crop Sci* 45:832–839
- Hobbie EA, Colpaert JV (2004) Nitrogen availability and mycorrhizal colonization influence water use efficiency and carbon isotope patterns in *Pinus sylvestris*. *New Phytol* 164:515–525
- Hose E, Clarkson DT, Steudle E, Schreiber L, Hartung W (2001) The exodermis: a variable apoplastic barrier. *J Exp Bot* 52:2245–2264
- Hubbard R, Ryan M, Stiller V, Sperry J (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant Cell Environ* 24:113–121
- Iiyama K, Wallis AFA (1990) Determination of lignin in herbaceous plants by an improved acetyl bromide procedure. *J Sci Food Agr* 51:145–161
- Ishikawa-Sakurai J, Hayashi H, Murai-Hatano M (2014) Nitrogen availability affects hydraulic conductivity of rice roots, possibly through changes in aquaporin gene expression. *Plant Soil* 379:289–300
- Javot H, Maurel C (2002) The role of aquaporins in root water uptake. *Ann Bot* 90:301–313
- Kaldenhoff R, Fischer M (2006) Functional aquaporin diversity in plants. *BBA Biomembranes* 1758:1134–1141
- Kirk GJD, Kronzucker HJ (2005) The potential for nitrification and nitrate uptake in the rhizosphere of wetland plants: a modelling study. *Ann Bot* 96:639–646
- Lajtha K, Whitford W (1989) The effect of water and nitrogen amendments on photosynthesis, leaf demography, and resource-use efficiency in *Larrea tridentata*, a desert evergreen shrub. *Oecologia* 80:341–348
- Lawlor DW (2002) Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *J Exp Bot* 53:773–787
- Li Y, Gao Y, Xu X, Shen Q, Guo S (2009) Light-saturated photosynthetic rate in high-nitrogen rice (*Oryza sativa* L.) leaves is related to chloroplastic CO₂ concentration. *J Exp Bot* 60:2351–2360
- Li Y, Ren B, Ding L, Shen Q, Peng S, Guo S (2013) Does chloroplast size influence photosynthetic nitrogen use efficiency? *PloS one* 8(4):e62036. doi:10.1371/journal.pone.0062036
- Linkohr BI, Williamson LC, Fitter AH, Leyser H (2002) Nitrate and phosphate availability and distribution have different effects on root system architecture of *Arabidopsis*. *Plant J* 29:751–760
- Livingston N, Guy R, Sun Z, Ethier G (1999) The effects of nitrogen stress on the stable carbon isotope composition, productivity and water use efficiency of white spruce (*Picea glauca* (Moench) Voss) seedlings. *Plant Cell Environ* 22:281–289
- Long SP, Bernacchi CJ (2003) Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *J Exp Bot* 54:2393–2401
- Long SP, Farage PK, Garcia RL (1996) Measurement of leaf and canopy photosynthetic CO₂ exchange in the field. *J Exp Bot* 47:1629–1642
- Lu Z, Neumann PM (1999) Water stress inhibits hydraulic conductance and leaf growth in rice seedlings but not the transport of water via mercury-sensitive water channels in the root. *Plant Physiol* 120:143–152
- Lux A, Morita S, Abe J, Ito K (2005) An improved method for clearing and staining free-hand sections and whole-mount samples. *Ann Bot* 96:989–996
- Maggio A, Joly RJ (1995) Effects of mercuric chloride on the hydraulic conductivity of tomato root systems (evidence for a channel-mediated water pathway). *Plant Physiol* 109:331–335
- Martre P, North GB, Nobel PS (2001) Hydraulic conductance and mercury-sensitive water transport for roots of *Opuntia acanthocarpa* in relation to soil drying and rewetting. *Plant Physiol* 126:352–362
- Maurel C, Verdoucq L, Luu DT, Santoni V (2008) Plant aquaporins: membrane channels with multiple integrated functions. *Annu Rev Plant Biol* 59:595–624
- Morgan JA (1986) The effects of N nutrition on the water relations and gas exchange characteristics of wheat (*Triticum aestivum* L.). *Plant Physiol* 80:52–58

- Nayyar H (2003) Accumulation of osmolytes and osmotic adjustment in water-stressed wheat (*Triticum aestivum*) and maize (*Zea mays*) as affected by calcium and its antagonists. *Environ Exp Bot* 50:253–264
- North GB, Nobel PS (1995) Hydraulic conductivity of concentric root tissues of *Agave deserti* Engelm. under wet and drying conditions. *New Phytol* 130:47–57
- North GB, Nobel PS (2000) Heterogeneity in water availability alters cellular development and hydraulic conductivity along roots of a desert succulent. *Ann Bot* 85:247–255
- Oddo E, Inzerillo S, La Bella F, Grisafi F, Salleo S, Nardini A (2011) Short-term effects of potassium fertilization on the hydraulic conductance of *Laurus nobilis* L. *Tree Physiol* 31:131–138
- Parent B, Hachez C, Redondo E, Simonneau T, Chaumont F, Tardieu F (2009) Drought and abscisic acid effects on aquaporin content translate into changes in hydraulic conductivity and leaf growth rate: a trans-scale approach. *Plant Physiol* 149:2000–2012
- Passioura J (2007) The drought environment: physical, biological and agricultural perspectives. *J Exp Bot* 58:113–117
- Pitre F, Cooke JK, Mackay J (2007) Short-term effects of nitrogen availability on wood formation and fibre properties in hybrid poplar. *Trees* 21:249–259
- Postma JA, Lynch JP (2011) Root cortical aerenchyma enhances the growth of maize on soils with suboptimal availability of nitrogen, phosphorus, and potassium. *Plant Physiol* 156:1190–1201
- Radin JW (1983) Control of plant growth by nitrogen: differences between cereals and broadleaf species. *Plant Cell Environ* 6:65–68
- Radin JW, Boyer JS (1982) Control of leaf expansion by nitrogen nutrition in sunflower plants role of hydraulic conductivity and turgor. *Plant Physiol* 69:771–775
- Saliendra NZ, Sperry JS, Comstock JP (1995) Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in *Betula occidentalis*. *Planta* 196:357–366
- Shangguan ZP, Shao MA, Dyckmans J (2000) Nitrogen nutrition and water stress effects on leaf photosynthetic gas exchange and water use efficiency in winter wheat. *Environ Exp Bot* 44:141–149
- Steudle E (2000a) Water uptake by plant roots: an integration of views. *Plant Soil* 226:45–56
- Steudle E (2000b) Water uptake by roots: effects of water deficit. *J Exp Bot* 51:1531–1542
- Steudle E (2001) The cohesion-tension mechanism and the acquisition of water by plant roots. *Annu Rev Plant Biol* 52:847–875
- Steudle E, Peterson CA (1998) How does water get through roots? *J Exp Bot* 49:775–788
- Tanguilig V, Yambao E, O'toole J, De Datta S (1987) Water stress effects on leaf elongation, leaf water potential, transpiration, and nutrient uptake of rice, maize, and soybean. *Plant Soil* 103:155–168
- Tezara W, Mitchell VJ, Driscoll SD, Lawlor DW (1999) Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature* 401:914–917
- Tian Q, Chen F, Zhang F, Mi G (2005) Possible involvement of cytokinin in nitrate-mediated root growth in maize. *Plant Soil* 277:185–196
- Vandeleur RK, Niemietz C, Tilbrook J, Tyerman SD (2005) Roles of aquaporins in root responses to irrigation. *Plant Soil* 274:141–161
- Vandeleur RK, Mayo G, Shelden MC, Gilliham M, Kaiser BN, Tyerman SD (2009) The role of plasma membrane intrinsic protein aquaporins in water transport through roots: diurnal and drought stress responses reveal different strategies between isohydric and anisohydric cultivars of grapevine. *Plant Physiol* 149:445–460
- Vander P, Vårum KM, Domard A, Eddine El Gueddari N, Moerschbacher BM (1998) Comparison of the ability of partially N-acetylated chitosans and chitooligosaccharides to elicit resistance reactions in wheat leaves. *Plant Physiol* 118:1353–1359
- Visser EJW, Colmer TD, Blom CWPM, Voeseek LACJ (2000) Changes in growth, porosity, and radial oxygen loss from adventitious roots of selected mono- and dicotyledonous wetland species with contrasting types of aerenchyma. *Plant Cell Environ* 23:1237–1245
- Wan X, Zwiazek JJ (1999) Mercuric chloride effects on root water transport in aspen seedlings. *Plant Physiol* 121:939–946
- Wan X, Steudle E, Hartung W (2004) Gating of water channels (aquaporins) in cortical cells of young corn roots by mechanical stimuli (pressure pulses): effects of ABA and of HgCl₂. *J Exp Bot* 55:411–422
- Wang JR, Hawkins CDB, Letchford T (1998) Photosynthesis, water and nitrogen use efficiencies of four paper birch (*Betula papyrifera*) populations grown under different soil moisture and nutrient regimes. *Forest Ecol Manag* 112:233–244
- Warren CR, Adams MA (2006) Internal conductance does not scale with photosynthetic capacity: implications for carbon isotope discrimination and the economics of water and nitrogen use in photosynthesis. *Plant Cell Environ* 29:192–201
- Welander NT, Ottosson B (2000) The influence of low light, drought and fertilization on transpiration and growth in young seedlings of *Quercus robur* L. *Forest Ecol Manag* 127:139–151
- Yang X, Li Y, Ren B, Ding L, Gao C, Shen Q, Guo S (2012) Drought-induced root aerenchyma formation restricts water uptake in rice seedlings supplied with nitrate. *Plant Cell Physiol* 53:495–504