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Higher nitrogen use efficiency (NUE) in hybrid “super rice” links to improved morphological and physiological traits in seedling roots

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ABSTRACT

Great progress has been achieved in developing hybrid “super rice” varieties in China. Understanding morphological root traits in super rice and the mechanisms of nitrogen acquisition by the root system are of fundamental importance to developing proper fertilisation and nutrient management practices in their production. The present study was designed to study morphological and physiological traits in hybrid super rice roots that are associated with nitrogen use efficiency (NUE). Two hybrid super rice varieties (Yongyou12, YY; Jiayou 6, JY) and one common variety (Xiushui 134, XS) with differing NUE were cultivated hydroponically, and morphological and physiological traits of seedling roots in response to varying nitrogen conditions were investigated. Our results show that the hybrid cultivars YY and JY exhibit larger root systems, arising from a maximisation of root tips and from longer roots without changes in root diameter. The cross-sectional proportion of aerenchyma was significantly higher in super rice roots. The larger root system of super hybrid rice contributed to higher N accumulation and resulted in higher N uptake efficiency. ^{15}N ($^{15}NH_4^+$) labeling results show that YY and JY had an enhanced capacity for ammonium (NH_4^+) uptake. Moreover, YY and JY were more tolerant to high NH_4^+ and showed reduced futile NH_4^+ efflux. NH_4^+ efflux in the root elongation zone, measured by Non-invasive Micro-test Technology, was significantly lower than in XS. Taken together, our results suggest that a longer root, a larger number of tips, a better developed aerenchyma, a higher capacity for N uptake, and reduced NH_4^+ efflux from roots are associated with higher NUE and growth performance in hybrid super rice.

1. Introduction

Continuing population growth and higher consumption demand for food place increasing strains upon global agricultural production (Godfray et al., 2010). Rice (*Oryza sativa*) is the dominant staple food for approximately half of the world’s population (Fageria, 2007). Increasing rice yield per unit area is crucial for improving agricultural land use efficiency and ensuring food security. In 1996, China initiated a “super rice" breeding program, focusing on hybrid rice lines, with the goal of producing high-yielding rice cultivars through morphological improvement and through the utilisation of inter-subspecific (indica/japonica) heterosis (Ma and Yuan, 2015; Yuan, 2017). Significant progress has been made over the past two decades, and many new “super rice” varieties have been developed. As of 2018, China had designated 176 super rice cultivars, defined by grain yield achievements of 12.0 ~ 16.0 t ha^{-1} in field production (Wei et al., 2018).

Due to morphological improvements and heterosis utilisation, coupled to high nitrogen (N) fertiliser applications, super hybrid rice cultivars display increased biomass, bigger panicle size, and larger sink size, manifesting in higher yield compared to common rice cultivars (Peng et al., 2008; Yuan, 2017; Zhang et al., 2009). Additionally, super rice cultivars usually have higher nitrogen use efficiency (NUE) as they display higher grain yield under identical nitrogen supply (Huang et al., 2018, 2015). Use of N-efficient cultivars is expected to not only increase grain yield but also reduce environmental cost. Super rice cultivars with high yield potential and high NUE have been planted increasingly throughout China. Understanding their root morphological traits and the mechanisms of N acquisition is important for the design of proper

Abbreviations: N, nitrogen; NH_4^+, ammonium; NO_3^-, nitrate; NUE, nitrogen use efficiency

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fertilisation and nutrient management practices in the field and for potential further improvements in cultivar genomes.

NUE is composed of N uptake efficiency and physiological N use efficiency (Duan et al., 2007). Previous studies have documented that total N uptake by plants is a function of root biomass, root morphology, root/plant growth rates, root proliferation, and the root's physiological capacity for N uptake (Glass, 2003). In rice, root physiological and morphological characteristics are closely associated with yield and NUE (Ju et al., 2015; Yang et al., 2012). There are reports showing that increases in grain yield and nutrient and water use efficiency were mainly attributed to root biomass, root length, root tips, and root-oxidizing activity (Mishra and Salokhe, 2011; Wu and Cheng, 2014). Well developed aerenchyma in rice roots is furthermore critical - it promotes radial oxygen transport from the atmosphere into the root rhizosphere, enhances rhizosphere oxygenation, and benefits plants experiencing deficiencies of nitrogen (Abiko and Obara, 2014; Kludze et al., 1993) as well as optimising the balance between reduced and oxidised N species in the rice rhizosphere (Kirk and Kronzucker, 2005; Kronzucker et al., 2000, 1999). Several studies have documented that root dry weight and root length density were significantly greater in hybrid super rice Liangyoupeijiu throughout the growing season (Zhang et al., 2009). However, root morphological characteristics, such as total length, average root diameter, root tip number, and aerenchyma formation in super rice, as well as how these traits relate to NUE, has remained unstudied.

For rice crops, ammonium (NH_4^+) and nitrate (NO_3^-) are the two main sources of inorganic nitrogen in paddy soil. NH_4^+ is a preferred nitrogen source, and is the main form of N available to the young rice plants (Britto and Kronzucker, 2002), but both N sources can be utilised and can occur in the rice rhizosphere, especially when aerenchyma production is pronounced (Kirk and Kronzucker, 2005; Kronzucker et al., 2000, 1999). N deficiency and NH_4^+ toxicity are widespread in rice production, inhibiting rice root growth and decreasing NUE (Di et al., 2018, Hirano et al., 2008; Li et al., 2010; Sun et al., 2017). The uptake of both NH_4^+ and NO_3^- is subject to down-regulation as tissue N levels approach an upper limit (Glass et al., 2002). Both constitutive high-affinity transport systems (HATS) and low-affinity transport systems (LATS) are present in the plasma membranes of rice root cells for NH_4^+ uptake. NH_4^+ efflux from roots results in a reduction of net N uptake, and the extent of efflux varies significantly depending on cultivars, external N concentration, and N status of the plant and the root tissue. NUE in rice is frequently associated with futile NH_4^+ cycling in roots, and larger NH_4^+ efflux in the elongation zone of the root has been observed in low-NUE cultivars under high NH_4^+ (Chen et al., 2013). However, the characteristics of NH_4^+ uptake of the root system and of NH_4^+ cycling across the plasma membrane of roots in super rice have not been explored.

In the present study, two hybrid super rice varieties and one common rice variety with differing NUE were examined in terms of the morphological and physiological traits of seedling roots under varying N conditions. We explored how the roots of super hybrid rices respond to low-nitrogen conditions and high NH_4^+ concentrations, and the extent to which the development of root aerenchyma is related to NUE. Furthermore, we investigated the characteristics of NH_4^+ uptake of the root system, and of NH_4^+ cycling across the plasma membrane of roots. Our study provides both theoretical and data support for the development of proper fertilisation and nutrient management practices in hybrid super rice production.

2. Materials and methods

2.1. Plant material, growth conditions, and experimental treatment

Three rice (*Oryza sativa* L.) cultivars were chosen as the experimental materials. One common variety was *japonica inbred* Xiushui 134 (XS). Two newly developed super rice hybrids were Yongyou12 (YY), Jiayou 6 (JY). Grain yield and NUE (kg grain yield kg^{-1} N applied) of these three rice cultivars were obtained from a previous field experiment (Table 1).

Table 1
Grain yield and NUE of the cultivars used in the present study.

	N applied ($kgNha^{-1}$)	XS	YY	JY
Grain yield($t\ ha^{-1}$)	200	9.10	11.15	11.81
	300	8.86	11.99	12.73
NUE($kg\ kg^{-1}$)	200	45.50	55.75	59.05
	300	29.53	39.97	42.43

Rice seeds were surface-sterilised with 10 % H_2O_2 for 30 min, rinsed thoroughly and soaked with deionized water for 24 h. The seeds were then germinated on floating nets in a culture box containing 0.5 mM $CaCl_2$. After 3 d of incubation at 30 °C in the dark, the germinated seeds were placed under light, supplied with 1/4-strength IRRI (International Rice Research Institute) rice nutrient solution (Yoshida et al., 1972). The full-strength nutrient solution contained macronutrients, as follows: NH_4NO_3, 1.43 mM; KH_2PO_4, 0.32 mM; K_2SO_4, 0.35 mM; $MgSO_4$, 1.65 mM; $CaCl_2$, 1.43 mM; $Na_2SiO_3{\cdot}9H_2O$, 0.1 mM; and micronutrients, as follows: $CuSO_4$, 0.16 μM; $MnCl_2$, 9.1 μM; $ZnSO_4$, 0.15 μM; H_3BO_3, 18.5 μM; $(NH_4)_6Mo_7O_{24}$, 0.52 μM; Na_2EDTA-Fe, 35.8 μM. Three days after, seedlings were supplied with half-strength IRRI rice nutrient solution. Four days later, three seedlings at a time were bundled and transplanted into a larger culture box, supplied with full-strength nutrient solution for one week. Subsequently, seedlings were supplied with different N conditions for two weeks.

Low-N conditions were 0.05 mM, 0.5 mM, and 1 mM, provided by 0.025, 0.25, 0.5 mM NH_4NO_3, respectively. The normal-N condition was 2.86 mM NH_4^+ provided as 1.43 mM $(NH_4)_2SO_4$, and the N concentration was consistent with the IRRI rice nutrient solution (Yoshida et al., 1972). As soil-solution NH_4^+ concentrations in paddy soil can easily fall into the 2–20 mM range (Glass et al., 2002), the high-NH_4^+ concentrations were set to 2, 5, 10, 15 mM, provided as 1, 2.5, 5, 7.5 mM $(NH_4)_2SO_4$, respectively.

Plants were grown in a controlled-environment chamber with a day/night temperature regime of 25 °C/28 °C, 65 % relative humidity, and a 14 -h light and 10 -h dark cycle. The light intensity was 400 μmol $m^{-2}\ s^{-1}$. The pH of the nutrient solution was maintained at 5.5 by adding diluted NaOH or HCl daily. The nutrient solution was exchanged completely every two days. Following hydroponic cultivation, 32-D-old seedlings were used for measurements.

2.2. Measurement of root morphology, dry weight, and nitrogen content

After treatment, 32-D-old hydroponically cultivated seedlings were harvested. Rice roots and shoots were separated and washed. Root morphology including total root length, root volume, root surface area, average root diameter, and root tip number in the three rice cultivars were measured using the root analysis instrument WinRhizo-LA1600 (Regent Instruments Inc., Quebec, Canada). Root and shoot samples were placed in a drying oven at 105 °C for 30 min, and dried to a constant weight at 70 °C. Then, the dry weights of roots and shoots were recorded. Samples were ground into powder and digested with H_2SO_4-H_2O_2 and the concentration of N was determined using the Kjeldahl method.

2.3. Evaluation of aerenchyma formation

Cross-sections of newly formed adventitious roots, approximately 60 mm long, were collected from 32-D-old seedlings in the three cultivars to evaluate aerenchyma formation by scanning electronic microscopy (SEM). For each cultivar, three adventitious roots from different plants were used for measurements. Roots were cut into 10-mm-long segments, from the 20 − 30-mm zone behind the root tip, using a razor blade. Subsequently, the cross-sections were fixed and vacuum-infiltrated with 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, for 3.5 h (Longstreth and Borkhsenious, 2000). Sections were then dehydrated in a graded ethanol series and dried using a critical point dryer (Quorum K850, England). The dried root sections were cut from the middle section, about 25 mm behind the root tip, using the double-edge razor blades, and glued onto specimen stubs. Then, the dried sections were viewed and photographed by SEM (FEI Quanta 2000, USA). The area of aerenchyma was calculated using Image J software (ver. 1.65, NIH, available at http://rsb.info.nih.gov/ij).

2.4. NH_4^+ uptake rate of the root system

NH_4^+ uptake was measured using an ^{15}N-labeling method. After pre-culturing for four weeks in IRRI rice nutrient solution (Yoshida et al., 1972), seedlings of XS, YY, and JY were selected for the determination of N-absorption kinetics. Following 2-d N starvation, seedlings of XS, YY, and JY were transferred to nutrient solution to which 0, 0.05, 0.2, 0.5, 1, and 3 mM of $^{15}NH_4NO_3$ (99.10 atom percent ^{15}N) were added. After three hours of treatment, rice roots were washed, separated, dried to a constant weight at 70 °C, ground into powder, and subjected to a Thermo Flash 2000 analyzer hyphenated to a Thermo Fisher (Waltham, MA, USA) Delta-V isotope ratio mass spectrometer to determine ^{15}N abundance.

2.5. Measurement of net NH_4^+ fluxes with the NMT system

After treatment in normal-N (2.86 mM NH_4^+) and high-N (15 mM NH_4^+) nutrient solution for two weeks, 32-D-old seedlings were collected. Net NH_4^+ fluxes in the elongation zone were measured using Non-invasive Micro-test Technology (Younger USA LLC, 186 Amherst, MA 01002, USA). The principle of this method and the instrument have been previously detailed (Chen et al., 2013). For each treatment, three to six plants were randomly selected for net NH_4^+ fluxes analyses. After roots were equilibrated in a Petri dish containing 10–20 mL of measuring solution (0.5 mM NH_4Cl, pH = 5.5) for 30 min, they were transferred to another small Petri dish containing 3 ml of fresh measuring solution. The ion-selective electrodes were calibrated using NH_4^+ concentrations of 0.2 mM and 2.0 mM. Microelectrodes were vibrated along an axis perpendicular to the root, to find the positions where the maximum net fluxes of NH_4^+ occurred. Then, net fluxes of NH_4^+ at the elongation zone were further investigated in detail. The recording rate for ion fluxes was one reading per 6 s, and each measurement point was monitored for 5 min.

2.6. Data analysis

Data were analyzed statistically by the statistical software program SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Means were compared using one-way analysis of variance (ANOVA) with Duncan's multiple-range test. Significant differences ($P < 5\%$) between treatments are indicated by different letters in the figure and table legends. Graphs were produced using Origin 8.5. All graphs and images were arranged using Adobe Photoshop 7.0.

3. Results

3.1. Root morphology under low-nitrogen conditions

To investigate root growth in hybrid super rice under low-nitrogen conditions, root morphology and root dry weight in XS, YY, and JY seedlings were measured. The results show that root morphology and root dry weight in the super rice cultivars YY and JY are different from those in the common variety (Fig. 1). Both YY and JY exhibited larger root systems compared with XS. Root dry weight of YY and JY was 50 % and 209 % higher than in XS at 1 mM, 9% and 48 % higher at 0.5 mM, and 19 % and 65 % higher at 0.05 mM, respectively. Root dry weight, root volume, root surface area of YY and JY increased in parallel with root total length and root tip number when N concentration was elevated. However, average root diameter in YY and JY was significantly lower than that in XS at 0.05 mM and 0.5 mM ($P < 0.05$). Root dry weight, total root length, root volume, root surface area, and root tip number in the three rice cultivars increased more rapidly as the N concentration increased from 0.5 mM to 1 mM than when N concentrations were elevated from 0.05 mM to 0.5 mM. Altogether, compared to the common variety XS, the super rice cultivars YY and JY exhibited better root growth under low-N conditions.

When N concentration was increased from 0.05 mM to 1.0 mM, dry weight and N accumulation in shoots increased rapidly, in parallel with root dry weight, and significantly better shoot growth was observed in the super rice cultivars YY and JY. Shoot dry weight of YY and JY was 18 % and 49 % higher than in XS at 0.05 mM, 37 % and 89 % higher at 0.5 mM, and 51 % and 205 % higher at 1 mM, respectively. N accumulation in shoots of YY and JY was 33 % and 57 % higher than in XS at 0.05 mM, 59 % and 116 % higher at 0.5 mM, and 103 % and 273 % higher at 1 mM, respectively. These data suggest that an improved root system contributes to better shoot growth and higher N accumulation (Figs. 2, S1).

3.2. Aerenchyma formation under normal nitrogen conditions

To explore aerenchyma formation in hybrid super rice roots, the radial-arrangement aerenchyma tissues in the three rice cultivars were examined. Aerenchyma formation was initiated in the 10 − 20-mm zone from the root tip and gradually increased toward the basal part of rice roots. Cross-sections at 25 mm behind adventitious root tips were viewed by SEM (Fig. 3) . The parenchyma cells of super rice cultivars YY and JY collapsed and the radial cell wall aggregated together to form a gas-filled space connecting the stele and the exodermis. Aerenchyma in root cross-sections taken at 25 mm behind adventitious root tips of XS, YY, and JY covered 21.86 %, 39.96 %, and 42.06 % of the root cross-sectional area, respectively (Fig. 4). The proportion of aerenchyma in the super rice cultivars YY and JY were significantly higher than in XS ($P < 0.05$).

3.3. Root morphology under high NH_4^+ conditions

NH_4^+ toxicity occurs when NH_4^+-release fertilizers such as urea are applied in excessive quantities in rice fields. To explore the tolerance to high NH_4^+, root morphological traits and root dry weight in the three rice cultivars were measured at 2, 5, 10 and 15 mM NH_4^+ (Fig. 5). Root dry weight, total root length, root volume, root surface area, and root tip number in the super rice cultivars YY and JY were significantly higher than those in XS at 2, 5, 10, and 15 mM NH_4^+ ($P < 0.05$). Root growth of YY and JY increased strongly with increasing NH_4^+ concentrations from 2 mM to 5 mM, and only decreased when NH_4^+ concentrations were elevated from 5 mM to 15 mM. By contrast, root growth of XS was inhibited progressively as NH_4^+ concentrations increased from 2 mM to 15 mM. Root dry weight of YY and JY increased by 14 % and 73 %, respectively, when NH_4^+ concentrations were elevated from 2 mM to 5 mM, while it decreased by 42 % in XS. This implies that the hybrid rice cultivars YY and JY have higher thresholds for NH_4^+ toxicity. No obvious differences in average root diameter were found among the NH_4^+ treatments, showing that elevated NH_4^+ concentrations had little effect on average root diameter.

Dry weight and N accumulation in shoots of YY and JY changed in parallel with root dry weight, and significant advantages over XS were observed under each treatment (Figs. 6, S2). Differences in N content of shoots among treatments in the three cultivars were not prominent. N accumulation in shoots of YY and JY increased by 36 % and 60 %, respectively, when NH_4^+ concentrations were elevated from 2 mM to 5

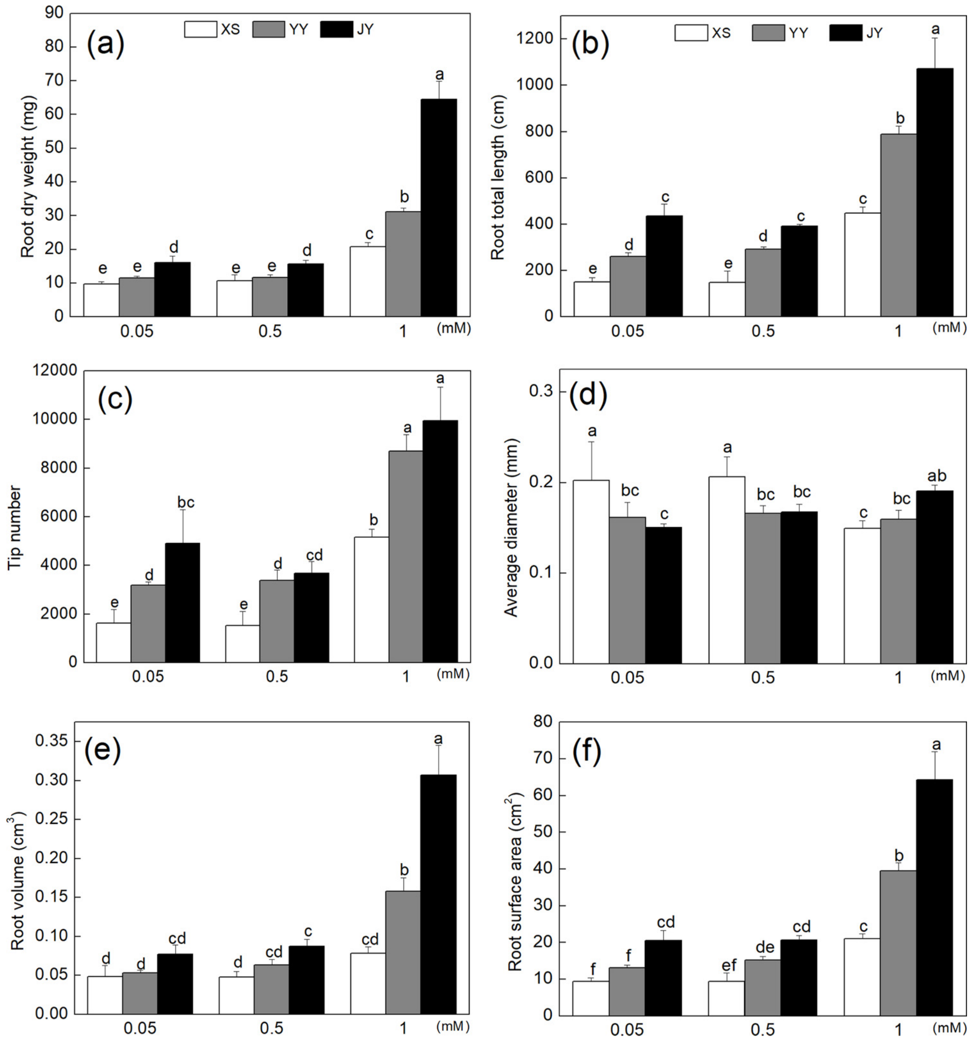

Fig. 1. Root dry weight (a), total length (b), tip number (c), average diameter (d), root volume (e), root surface area, (f) in XS, YY, and JY seedlings supplied with 0.05, 0.5 and 1 mM N. Values shown are the means ± SD of three replicates. For each datum, bars with different letters indicate significant differences (Duncan's test, at P < 0.05).

mM, while XS decreased by 46 %.

3.4. NH_4^+ uptake kinetics of the root system

NH_4^+ uptake rate of the rice roots was analyzed using ^{15}N ($^{15}NH_4NO_3$) labeling. In this concentration range, there was clear evidence of Michaelis–Menten-style saturation kinetics for NH_4^+ uptake in all three rice cultivars (Fig. 7). Both super rice cultivars YY and JY showed a significantly higher capacity for NH_4^+ uptake: maximum uptake rate (V_{max}) in YY and JY was 70 % and 60 % higher than in XS, respectively (Table 2). The Michaelis constant (*Km*) for NH_4^+ uptake in YY and JY was 123 %, and 91 % higher than in XS, respectively. This suggests that the roots of YY and JY possess a higher capacity for NH_4^+ uptake but that root transport systems in XS possess higher affinity for NH_4^+.

3.5. Net NH_4^+ fluxes in the elongation zone of rice roots

Net NH_4^+ fluxes at the surface of the elongation zones in roots of the three rice cultivars were measured by the non-invasive micro-test technique (Fig. 8). The roots of both YY and JY showed NH_4^+ influx primarily in the elongation zone, while the roots of XS showed weak efflux under normal (control) N conditions. Net NH_4^+ influx in roots of YY and JY was 138 pmol cm^{-2} s^{-1} and 116 pmol cm^{-2} s^{-1}, respectively, and net NH_4^+ efflux in XS was 35 pmol cm^{-2} s^{-1}. High NH_4^+ changed the direction of NH_4^+ flux in the elongation zones of YY and JY, and increased NH_4^+ efflux in XS. NH_4^+ efflux was detected in the elongation zones of both YY and JY when supplied with high NH_4^+. Net NH_4^+ efflux in YY and JY was 13 pmol cm^{-2} s^{-1}, and 18 pmol cm^{-2} s^{-1}, respectively, significantly lower than the 127 pmol cm^{-2} s^{-1} observed in XS. The results show that the hybrid super rice varieties YY and JY have greatly enhanced and more efficient uptake of NH_4^+ in their root system.

4. Discussion

4.1. Hybrid super rice cultivars' larger root system is attributable to a greater number of root tips and root length but not alterations in root diameter

During the last two decades, many new super rice varieties with higher yield potential and higher NUE have been developed. Prior to this study, limited work has focused on root morphology and the mechanisms of N acquisition by the root systems of newly developed hybrid super rice cultivars under varying nitrogen conditions in the seedling stage. Results from the present study provide evidence that hybrid super rice cultivars exhibit a much enlarged root system, which is predominantly attributable to a greater number of root tips and

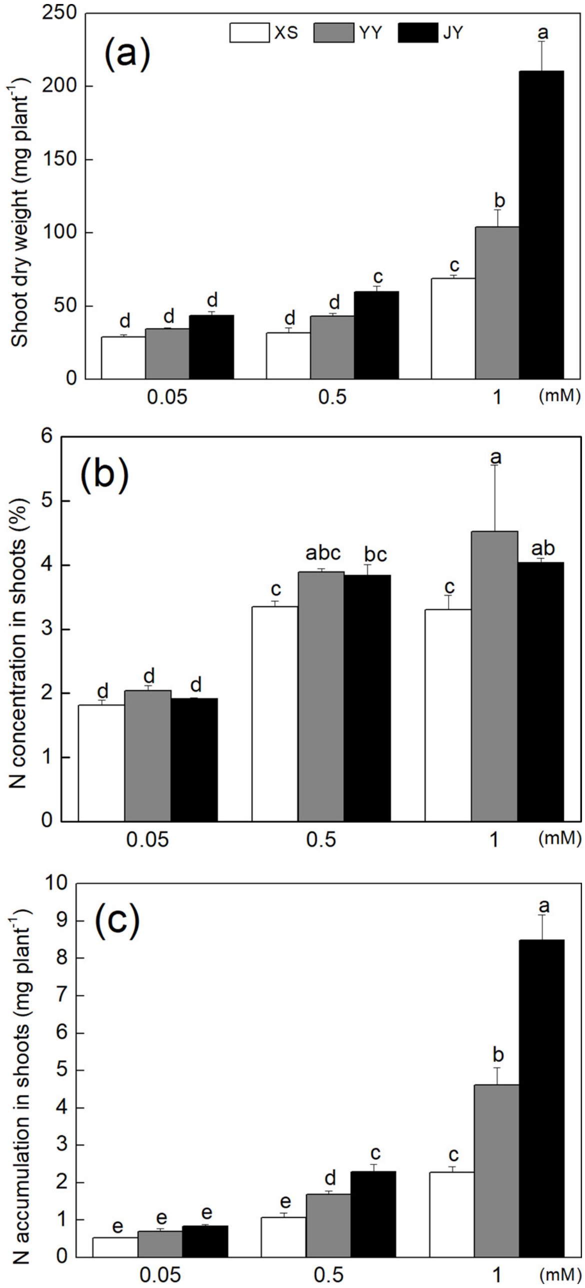

Fig. 2. Dry weight, N concentration, and N accumulation in shoots of XS, YY, and JY seedlings supplied with 0.05, 0.5 and 1 mM N. Values shown are the means ± SD of three replicates. For each datum, bars with different letters indicate significant differences (Duncan's test, at $P < 0.05$).

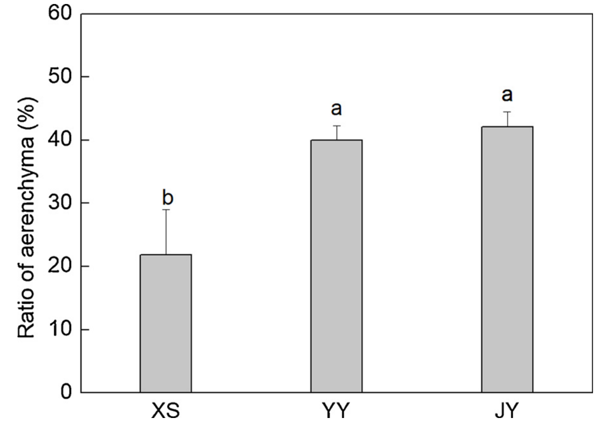

Fig. 4. Proportion of aerenchyma in adventitious roots of three rice varieties 25 mm behind the root tip. Values shown are the means ± SD of three replicates. For each datum, bars with different letters indicate significant differences (Duncan's test, at $P < 0.05$).

longer roots but not changes in root diameter. A Longer roots and a larger number of root tips enable rice plants to access a greater volume of soil and to acquire more nutrients from various depths. This finding is consistent with field studies that show larger root biomass, deeper root distribution, and larger root length contribute to higher grain yield and higher NUE (Ju et al., 2015).

Under low N conditions (0.05 mM and 0.5 mM) and high NH_4^+ conditions (10 mM and 15 mM NH_4^+), root growth was inhibited in all three cultivars, as were shoot growth (incl. shoot dry weight) and N accumulation in shoots. Shoot N accumulation was positively correlated with root traits, and the larger root system in the hybrid super rice cultivars clearly contributed to greater biomass and higher N accumulation. In rice at the vegetative stage, NUE is composed of N uptake efficiency (N accumulation relative to supply) and physiological N use efficiency (biomass accumulation relative to N accumulation) (Shi et al., 2010). Our results suggest that higher NUE in hybrid super rice cultivars is mainly related to higher N-uptake efficiency. Excessive N decreased N accumulation and NUE. This is consistent with previous a field study that showed that moderate nitrogen treatments stimulated root growth, and that excessive N application rates resulted in a lower yield in hybrid super rice (Liu et al., 2018).

4.2. *Enhanced aerenchyma formation contributes to high NUE*

The degree of oxygenation of the root rhizosphere is vital for plant root function. An increase in rhizospheric oxygen concentrations significantly enhances metabolic root activity and the plant's capacity for detoxification (Niu et al., 2012). In rice roots, aerenchyma is formed constitutively and its formation is enhanced under oxygen-deficient conditions (Drew et al., 2000; Yamauchi et al., 2013). Inducible

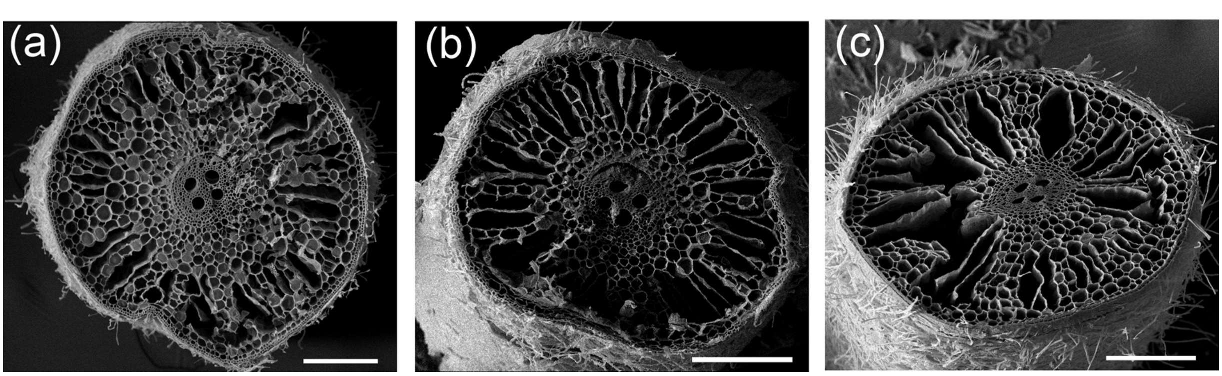

Fig. 3. SEM micrographs of transverse sections of XS (a), YY (b), and JY (c) 25 mm behind the root tip. Bars = 200 μm.

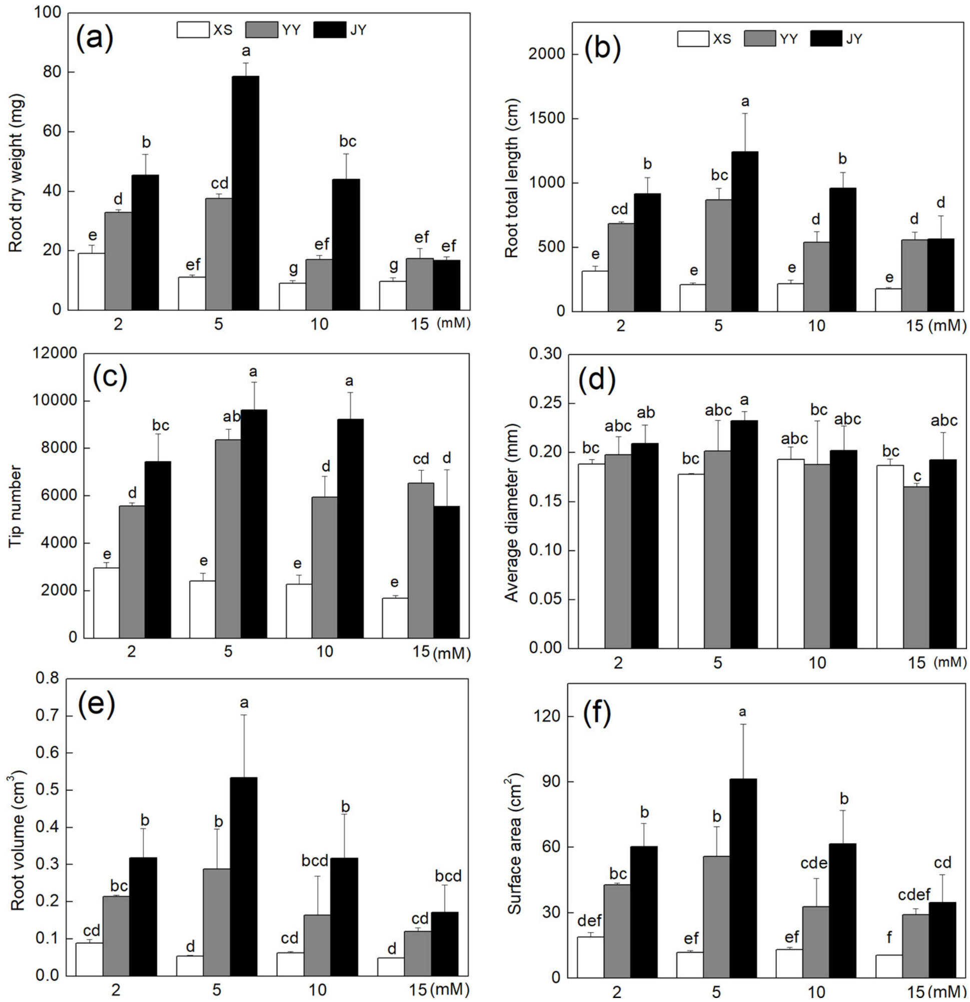

Fig. 5. Root dry weight (a), total length (b), tip number (c), average diameter (d), root volume (e), root surface area, (f) in XS, YY and JY seedlings supplied with 2, 5, 10 and 15 mM NH_4^+. Values shown are the means ± SD of three replicates. Different letters represent significant differences (Duncan's test, at P < 0.05).

aerenchyma formation in response to oxygen deficiency was evaluated at oxygen concentrations less than 1.0 mg/l (Shiono et al., 2011). The dissolved oxygen in our experiment was not in the "deficient: range, as dissolved oxygen concentrations in solution were at least 4.8 mg/L. It was observed that the proportion of aerenchyma in adventitious roots of the hybrid super rice cultivars YY and JY was significantly higher than that in XS. A well developed aerenchyma system promotes oxygen transport from shoots to root tips and radial oxygen loss from the roots to the rhizosphere, rhizospheric oxygen concentrations increase which help maintain aerobic microbial processes, such as the conversion of NH_4^+ into NO_3^- by nitrifying bacteria (Kludze et al., 1993; Li et al., 2008). Better developed aerenchyma in rice roots resulted in larger population of ammonia-oxidizing bacteria living in the rhizosphere, which in turn, directly enhance the nitrification activity in the rhizosphere (Ghosh and Kashyap, 2003). The co-presence of NO_3- then raise the thresholds of toxic NH_4^+ concentration and reduce NH_4^+ toxicity, because NO_3- uptake is associated with alkalinization of the root medium and stimulation of cation uptake, counteracting some of the effects of NH_4^+ (Li et al., 2014). A previous study demonstrated that enhanced aerenchyma were observed under nitrogen-deficient conditions (Abiko and Obara, 2014), and that greater N uptake of a high-NUE cultivar could be mainly attributed to an enhanced capacity for rhizosphere nitrification in rice plants (Li et al., 2008). Better developed aerenchyma in the hybrid super rice cultivars YY and JY could reduce cellular maintenance costs, promote internal oxygen transport, recycle nutrients, and facilitate rhizosphere oxygenation and nitrification. Better developed aerenchyma in hybrid super rice roots is one of the contributors to greater N uptake, and has beneficial effects on NUE. The importance of oxygen extrusion via aerenchyma in hypoxic to anoxic rice paddy soils for optimised nitrogen speciation (the balance between non-nitrified ammonium and nitrate, achieved by nitrification in the rhizosphere) has been demonstrated previously (Kirk and Kronzucker, 2005; Kronzucker et al., 2000, 1999).

4.3. Hybrid super rice cultivars possess a higher capacity for NH_4^+ uptake

The capacity of dealing with NH_4^+ is closely relates to rice growth and yield. It is well know that roots can change patterns of expression and regulation of membrane-bound transport proteins to facilitate acclimation to different N concentrations in soil, and such expression and post-transcriptional adjustments occur both in high-affinity transport systems (HATS) and in low-affinity transport systems (LATS) for NH_4^+ uptake (Britto and Kronzucker, 2006; Lea and Azevedo, 2006). Using an ^{15}N ($^{15}NH_4^+$) labeling method, it was observed that maximum uptake rates (V_{max}) and Michaelis constants (Km) in the hybrid super rice lines YY and JY were significantly higher than those in XS. V_{max} is typically reflective of the number of ion transporters present in cell membranes, while Km describes the affinity of the transporters for the ions in question (Duan et al., 2007; Kronzucker et al., 1995a, 1996, Kronzucker et al., 1995b), and the improved NH_4^+ uptake in the super rice cultivars can therefore reasonably be attributed to an increased number of NH_4^+ carriers. Roots of YY and JY had a higher capacity for NH_4^+

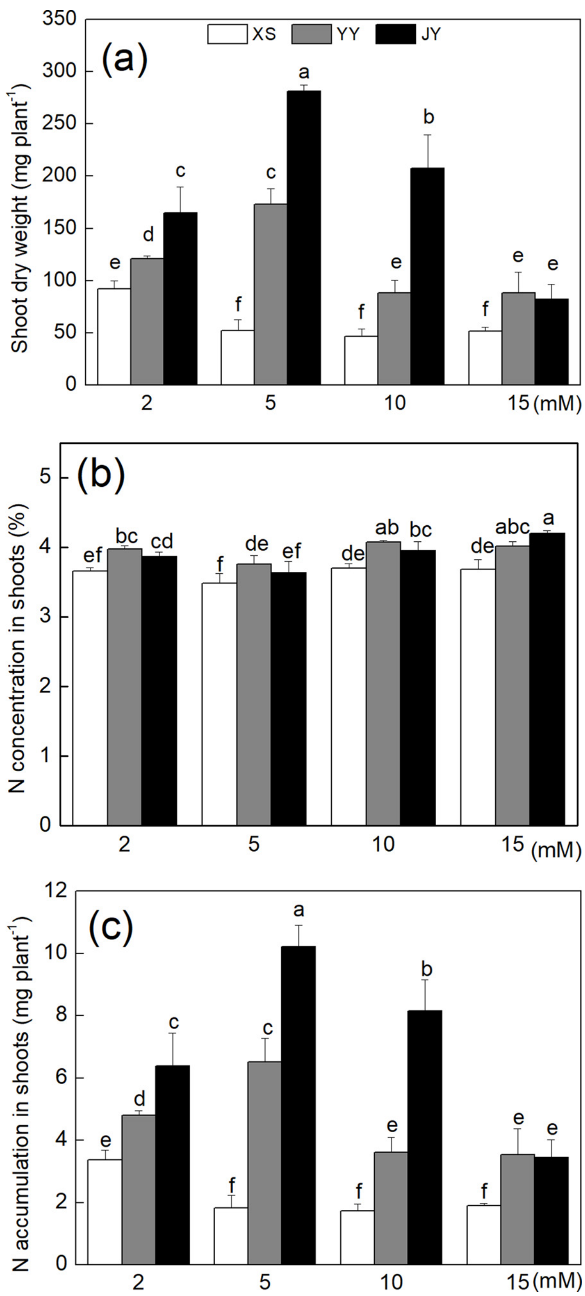

Fig. 6. Dry weight, N concentration, and N accumulation in shoots of XS, YY, and JY seedlings supplied with 2, 5, 10, and 15 mM NH_4^+. Values shown are the means ± SD of three replicates. Different letters represent significant differences (Duncan's test, at $P < 0.05$).

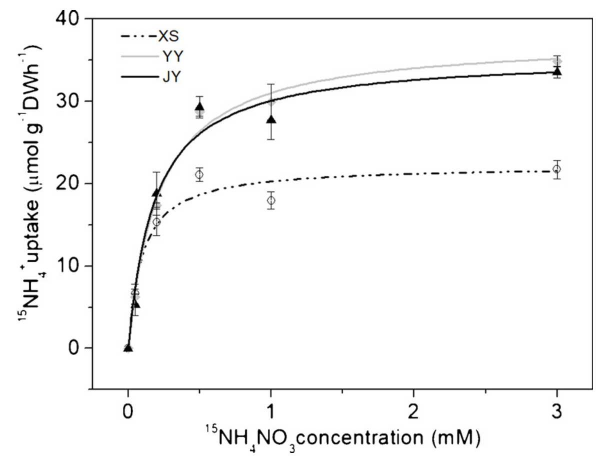

Fig. 7. $^{15}NH_4^+$ uptake in roots of XS, YY, and JY, in 32-D-old seedlings. Values shown are the means ± SD of three replicates, and are fitted to the Michaelis–Menten equation. DW: dry weight.

uptake, but XS displayed a higher affinity for NH_4^+. The hybrid super rice cultivars YY and JY appear to be better adapted to high-nitrogen conditions, while XS appears better adapted to low-nitrogen conditions.

4.4. Hybrid super rice cultivars are more tolerant to high NH_4^+ and this correlates with reduced NH_4^+ efflux from roots

High concentrations of NH_4^+ supplied to rice roots as the sole nitrogen are toxic to most plants (Britto and Kronzucker, 2002), including rice, although the toxicity threshold in rice tends to be higher than in most other agriculturally important plants (Balkos et al., 2010; Britto et al., 2014; Chen et al., 2013; Li et al., 2014; Sun et al., 2017). Our current study shows that hybrid super rice cultivars possess a higher threshold for NH_4^+ toxicity than commonly used varieties. NH_4^+ influx is mediated by energetically active high-affinity transport systems (HATS) in the plasma membranes of root cells at low NH_4^+ concentrations, and by high-capacity, energetically passive, low-affinity transport systems (LATS) under high NH_4^+ concentrations (Britto and Kronzucker, 2006; Chen et al., 2013), and this involves both members of the AMT family of transporters (Ludewig et al., 2007; Rawat et al., 1999; von Wiren et al., 2000) and members of the aquaporin (AQP) family of transporters (Coskun et al., 2013; Kronzucker and Britto, 2011; Loque et al., 2005; Saparov et al., 2007). As reported by Li et al. (2010) in Arabidopsis, cell elongation was the principal target in the NH_4^+ inhibition of primary root growth, and inhibition of cell elongation was associated with NH_4^+ efflux in the root elongation zone, the elongation zone was more sensitive to NH_4^+ stress than maturation zone. Chen et al. (2013) found that NUE in rice was associated with NH_4^+ cycling and tissue accumulation in the elongation zone of the root. Based on these findings, we measured NH_4^+ fluxes at the surface of the elongation zone of roots in the three selected rice cultivars. Under moderate NH_4^+ levels, significantly larger NH_4^+ influxes were detected at the elongation zones in YY and JY, while NH_4^+ effluxes were detected in XS. At high NH_4^+ concentrations, the direction of NH_4^+ flux in YY and JY, however, switched, and NH_4^+ efflux was stimulated in the elongation zone. These observations illustrate that the onset of futile efflux, typically associated with ammonium toxicity (Britto et al., 2001), in YY and JY is significantly higher than in XS; elevated NH_4^+ efflux in the elongation zone is energy-demanding and results in inhibition of root elongation. These findings show that hybrid super rice cultivars possess greater capacity to tolerate elevated NH_4^+ in the seedling stage and that this is associated with improved regulation of futile NH_4^+ cycling.

5. Conclusions

The present study examined morphological and physiological traits in roots of hybrid super rice that link to NUE. Our findings show that the hybrid super rice cultivars YY and JY have larger root systems, characterized by a higher number of tips and longer roots, but not greater root diameter. The proportion of aerenchyma in hybrid super rice roots is as well significantly higher. Under low and moderate N conditions, higher NUE in hybrid super rice cultivars is mainly attributed to larger root system, better developed aerenchyma, and a higher capacity for N uptake. Under high-N conditions, hybrid super rice cultivars maintain root growth by regulating futile NH_4^+ cycling in their root elongation zone, resulting in higher NUE. We suggest that these root traits be taken into account in breeding of N-efficient rice cultivars. Our findings should also be of utility in guiding fertilisation

Table 2
Kinetics parameters of $^{15}NH_4^+$ uptake in XS, YY, and JY seedlings (means ± SD, n = 3). V_{max} and K_m were determined by a non-linear curve-fitting model (Michaelis–Menten kinetics). DW: dry weight.

	Cultivar	Kinetic equation	R^2	K_m(μmol/L)	V_{max}(μmol g^{-1} DW h^{-1})
Common variety	XS	v = 22.23*[C]/(0.09625 + [C])	0.9428	96.25 ± 20.04 [b]	22.23 ± 0.94 [b]
Hybrid super rice	YY	v = 37.68*[C]/(0.2148 + [C])	0.9865	214.8 ± 21.18 [a]	37.68 ± 0.96[a]
	JY	v = 35.59*[C]/(0.1834 + [C])	0.9611	183.4 ± 31.09 [a]	35.59 ± 1.49 [a]

Different letters in the same column represent significant differences (at P < 0.05).

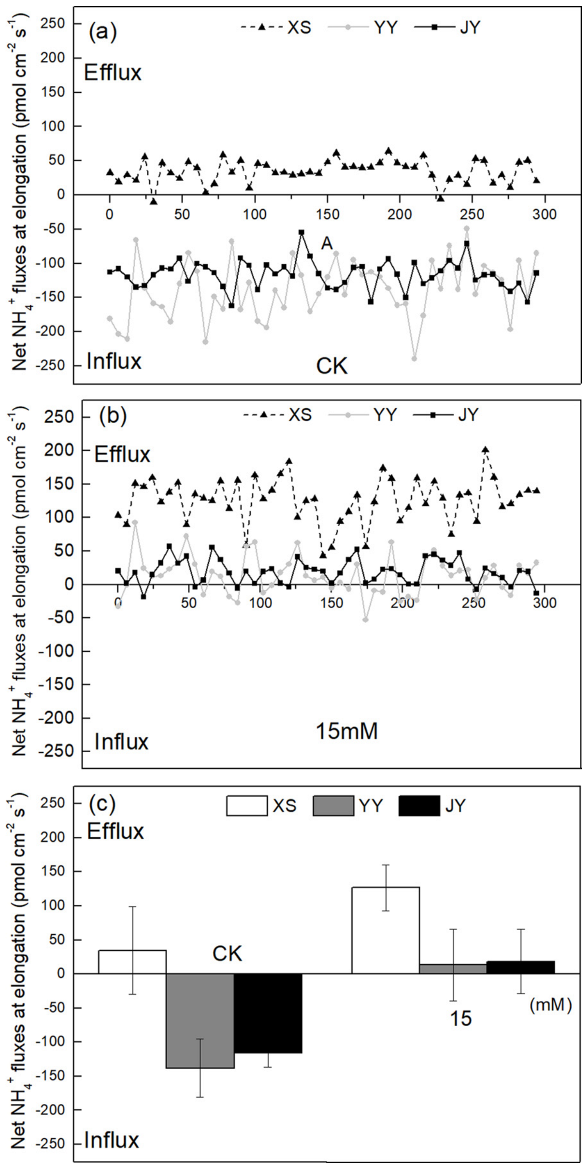

Fig. 8. Net plasma-membrane NH_4^+ fluxes in XS, YY, and JY rice varieties supplied with normal N (2.86 mM NH_4^+) and high N (15 mM NH_4^+) in the elongation zone (a, b), and mean values of NH_4^+ fluxes from (a, b).

and nutrient management practices, and thus super rice growth and yield performance, in the field.

Authors' contributions

MC, GC and WMS conceived and designed the research; MC carried out the experiments and performed the analyses; MC, GC, DWD, HJK and WMS wrote the manuscript. All authors read and approved the final manuscript.

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CRediT authorship contribution statement

Mei Chen: Conceptualization, Methodology, Investigation, Data curation, Writing - original draft, Visualization. **Gui Chen:** Methodology, Validation, Resources. **Dongwei Di:** Visualization, Writing - review & editing. **Herbert J. Kronzucker:** Writing - review & editing. **Weiming Shi:** Conceptualization, Project administration, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jplph.2020.153191.

References

Abiko, T., Obara, M., 2014. Enhancement of porosity and aerenchyma formation in nitrogen-deficient rice roots. Plant Sci. 215-216, 76–83.
Balkos, K.D., Britto, D.T., Kronzucker, H.J., 2010. Optimization of ammonium acquisition and metabolism by potassium in rice (Oryza sativa L. cv. IR-72). Plant Cell Environ. 33, 23–34.
Britto, D.T., Kronzucker, H.J., 2002. NH_4^+ toxicity in higher plants: a critical review. J. Plant Physiol. 159, 567–584.
Britto, D.T., Kronzucker, H.J., 2006. Futile cycling at the plasma membrane: a hallmark of low-affinity nutrient transport. Trends Plant Sci. 11, 529–534.
Britto, D.T., Glass ADM, Kronzucker H.J., Siddiqi, M.Y., 2001. Cytosolic concentrations and transmembrane fluxes of NH_4^+/NH_3. An evaluation of recent proposals. Plant Physiol. 125, 523–526.
Britto, D.T., Balkos, K.D., Becker, A., Coskun, D., Huynh, W.Q., Kronzucker, H.J., 2014. Potassium and nitrogen poising: Physiological changes and biomass gains in rice and barley. Can. J. Plant Sci. 94, 1085–1089.
Chen, G., Guo, S., Kronzucker, H.J., Shi, W., 2013. Nitrogen use efficiency (NUE) in rice links to NH_4^+ toxicity and futile NH_4^+ cycling in roots. Plant Soil 369, 351–363.
Coskun, D., Britto, D.T., Li, M., Becker, A., Kronzucker, H.J., 2013. Rapid Ammonia gas transport accounts for futile transmembrane cycling under NH_3/NH_4^+ toxicity in plant roots. Plant Physiol. 163, 1859–1867.
Di, D.-W., Sun, L., Zhang, X., Li, G., Kronzucker, H.J., Shi, W., 2018. Involvement of auxin

in the regulation of ammonium tolerance in rice (Oryza sativa L.). Plant Soil 432, 373–387.
Drew, M.C., He, C.-J., Morgan, P.W., 2000. Programmed cell death and aerenchyma formation in roots. Trends Plant Sci. 5, 123–127.
Duan, Y.H., Zhang, Y.L., Ye, L.T., Fan, X.R., Xu, G.H., Shen, Q.R., 2007. Responses of rice cultivars with different nitrogen use efficiency to partial nitrate nutrition. Ann. Bot. 99, 1153–1160.
Fageria, N., 2007. Yield physiology of rice. J. Plant Nutr. 30, 843–879.
Ghosh, P., Kashyap, A.K., 2003. Effect of rice cultivars on rate of N-mineralization, nitrification and nitrifier population size in an irrigated rice ecosystem. Appl. Soil Ecol. 24, 27–41.
Glass, A.D.M., 2003. Nitrogen use efficiency of crop plants: physiological constraints upon nitrogen absorption. Crit Rev Plant Sci. 22, 453–470.
Glass, A.D., Britto, D.T., Kaiser, B.N., Kinghorn, J.R., Kronzucker, H.J., Kumar, A., et al., 2002. The regulation of nitrate and ammonium transport systems in plants. J. Exp. Bot. 53, 855–864.
Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., et al., 2010. Food security: the challenge of feeding 9 billion people. Science 327, 812–818.
Hirano, T., Satoh, Y., Ohki, A., Takada, R., Arai, T., Michiyama, H., 2008. Inhibition of ammonium assimilation restores elongation of seminal rice roots repressed by high levels of exogenous ammonium. Physiol. Plant. 134, 183–190.
Huang, M., Chen, J., Cao, F., Jiang, L., Zou, Y., Deng, G., 2015. Improving physiological N-use efficiency by increasing harvest index in rice: a case in super-hybrid cultivar Guiliangyou 2. Arch Agron Soil Sci. 62, 725–743.
Huang, L., Sun, F., Yuan, S., Peng, S., Wang, F., 2018. Different mechanisms underlying the yield advantage of ordinary hybrid and super hybrid rice over inbred rice under low and moderate N input conditions. Field Crops Res. 216, 150–157.
Ju, C., Buresh, R.J., Wang, Z., Zhang, H., Liu, L., Yang, J., et al., 2015. Root and shoot traits for rice varieties with higher grain yield and higher nitrogen use efficiency at lower nitrogen rates application. Field Crops Res. 175, 47–55.
Kirk, G., Kronzucker, H., 2005. The potential for nitrification and nitrate uptake in the rhizosphere of wetland plants: a modelling study. Ann. Bot. 96, 639.
Kludze, H., DeLaune, R., Patrick, W., 1993. Aerenchyma formation and methane and oxygen exchange in rice. Soil Sci. Soc. Am. J. 57, 386–391.
Kronzucker, H.J., Britto, D.T., 2011. Sodium transport in plants: a critical review. New Phytol. 189, 54–81.
Kronzucker, H.J., Siddiqi, M.Y., Glass, A., 1995a. Analysis of 13NH4+ efflux in spruce roots (A test case for phase identification in compartmental analysis). Plant Physiol. 109, 481–490.
Kronzucker, H.J., Yaeesh Siddiqi, M., Glass, A.D.M., 1995b. Compartmentation and flux characteristics of ammonium in spruce. Planta. 196, 691–698.
Kronzucker, H.J., Siddiqi, M.Y., Glass, A., 1996. Kinetics of NH_4^+ influx in spruce. Plant Physiol. 110, 773–779.
Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M., Kirk, G.J.D., 1999. Nitrate-ammonium synergism in Rice. A subcellular flux analysis. Plant Physiol. 119, 1041–1046.
Kronzucker, H.J., Glass ADM, Siddiqi M.Y., Kirk, G.J.D., 2000. Comparative kinetic analysis of ammonium and nitrate acquisition by tropical lowland rice: implications for rice cultivation and yield potential. New Phytol. 145, 471–476.
Lea, P.J., Azevedo, R.A., 2006. Nitrogen use efficiency. 1. Uptake of nitrogen from the soil. Ann. Appl. Biol. 149, 243–247.
Li, Y.L., Fan, X.R., Shen, Q.R., 2008. The relationship between rhizosphere nitrification and nitrogen-use efficiency in rice plants. Plant Cell Environ. 31, 73–85.
Li, Q., LI, B.H., Kronzucker, H.J., SHI, W.M., 2010. Root growth inhibition by NH_4^+ in Arabidopsis is mediated by the root tip and is linked to NH4+ efflux and GMPase activity. Plant Cell Environ. 33, 1529–1542.
Li, B., Li, G., Kronzucker, H.J., Baluska, F., Shi, W., 2014. Ammonium stress in Arabidopsis: signaling, genetic loci, and physiological targets. Trends Plant Sci. 19, 107–114.
Liu, K., He, A., Ye, C., Liu, S., Lu, J., Gao, M., et al., 2018. Root morphological traits and spatial distribution under different nitrogen treatments and their relationship with grain yield in super hybrid rice. Sci. Rep. 8.
Longstreth, D.J., Borkhsenious, O.N., 2000. Root cell ultrastructure in developing aerenchyma tissue of three wetland species. Ann. Bot. 86, 641–646.
Loque, D., Ludewig, U., Yuan, L.X., von Wiren, N., 2005. Tonoplast intrinsic proteins AtTIP2;1 and AtTIP2;3 facilitate NH3 transport into the vacuole. Plant Physiol. 137, 671–680.
Ludewig, U., Neuhauser, B., Dynowski, M., 2007. Molecular mechanisms of ammonium transport and accumulation in plants. FEBS Lett. 581, 2301–2308.
Ma, G.-h, Yuan, L.-p, 2015. Hybrid rice achievements, development and prospect in China. J. Integr. Agric. 14, 197–205.
Mishra, A., Salokhe, V.M., 2011. Rice root growth and physiological responses to SRI water management and implications for crop productivity. Paddy Water Environ. 9, 41–52.
Niu, W.Q., Jia, Z.X., Zhang, X., Shao, H.B., 2012. Effects of soil rhizosphere aeration on the root growth and water absorption of tomato. CLEAN–Soil, Air, Water. 40, 1364–1371.
Peng, S., Khush, G.S., Virk, P., Tang, Q., Zou, Y., 2008. Progress in ideotype breeding to increase rice yield potential. Field Crops Res. 108, 32–38.
Rawat, S.R., Silim, S.N., Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M., 1999. AtAMT1 gene expression and NH4+ uptake in roots of Arabidopsis thaliana: evidence for regulation by root glutamine levels. Plant J. 19, 143–152.
Saparov, S.M., Liu, K., Agre, P., Pohl, P., 2007. Fast and selective ammonia transport by aquaporin-8. J. Biol. Chem. 282, 5296–5301.
Shi, W.M., Xu, W.F., Li, S.M., Zhao, X.Q., Dong, G.Q., 2010. Responses of two rice cultivars differing in seedling-stage nitrogen use efficiency to growth under low-nitrogen conditions. Plant Soil 326, 291–302.
Shiono, K., Ogawa, S., Yamazaki, S., Isoda, H., Fujimura, T., Nakazono, M., et al., 2011. Contrasting dynamics of radial O2-loss barrier induction and aerenchyma formation in rice roots of two lengths. Ann. Bot. 107, 89–99.
Sun, L., Di, D., Li, G., Kronzucker, H.J., Shi, W., 2017. Spatio-temporal dynamics in global rice gene expression (Oryza sativa L.) in response to high ammonium stress. J. Plant Physiol. 212, 94–104.
von Wiren, N., Gazzarrini, S., Gojon, A., Frommer, W.B., 2000. The molecular physiology of ammonium uptake and retrieval. Curr. Opin. Plant Biol. 3, 254–261.
H-y, Wei, Zhu, Y., Qiu, S., Han, C., Hu, L., Xu, D., et al., 2018. Combined effect of shading time and nitrogen level on grain filling and grain quality in japonica super rice. J. Integr. Agric. 17, 2405–2417.
Wu, W., Cheng, S., 2014. Root genetic research, an opportunity and challenge to rice improvement. Field Crops Res. 165, 111–124.
Yamauchi, T., Shimamura, S., Nakazono, M., Mochizuki, T., 2013. Aerenchyma formation in crop species: a review. Field Crops Res. 152, 8–16.
Yang, J.-c, Zhang, H., Zhang, J.-h, 2012. Root morphology and physiology in relation to the yield formation of rice. J. Integr. Agric. 11, 920–926.
Yoshida, S., Forno, D.A., Cock, J.H., 1972. Laboratory Manual for Physiological Studies of Rice. International Rice Research Institute, Philippines 56pp.
Yuan, L., 2017. Progress in super-hybrid rice breeding. Crop J. 5, 100–102.
Zhang, H., Xue, Y., Wang, Z., Yang, J., Zhang, J., 2009. Morphological and physiological traits of roots and their relationships with shoot growth in "super" rice. Field Crops Res. 113, 0–40.