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

Mei Chena,b,c, Gui Chenb, Dongwei Di, Herbert J. Kronzucker e, Weiming Shi b,*

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., 2020; Wei et al., 2018). 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
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 Lianyoupeijiu 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 understudied.

For rice crops, ammonium (NH₄⁺) and nitrate (NO₃⁻) are the two main sources of inorganic nitrogen in paddy soil. NH₄⁺ 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 NH₄⁺ toxicity are widespread in rice production, inhibiting 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 NH₄⁺ and NO₃⁻ 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 NH₄⁺ uptake. NH₄⁺ 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 NH₄⁺ cycling in roots, and larger NH₄⁺ efflux in the elongation zone of the root has been observed in low-NUE cultivars under high NH₄⁺ (Chen et al., 2013). However, the characteristics of NH₄⁺ uptake of the root system and of NH₄⁺ 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 rice respond to low-nitrogen conditions and high NH₄⁺ concentrations, and the extent to which the development of root aerenchyma is related to NUE. Furthermore, we investigated the characteristics of NH₄⁺ uptake of the root system, and of NH₄⁺ 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), Jiyao 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).

Rice seeds were surface-sterilised with 10 % H₂O₂ 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 CaCl₂. 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: NH₄NO₃, 1.43 mM; KH₂PO₄, 0.32 mM; K₂SO₄, 0.35 mM; MgSO₄, 1.65 mM; CaCl₂, 1.43 mM; NaNO₃-9H₂O, 0.1 mM; and micronutrients, as follows: CuSO₄, 0.16 μM; MnCl₂, 9.1 μM; ZnSO₄, 0.15 μM; H₂BO₃, 18.5 μM; (NH₄)₂MoO₄·24, 0.52 μM; Na₂EDTA·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 NH₄NO₃, respectively. The normal-N condition was 2.86 mM NH₄⁺ provided as 1.43 mM (NH₄)₂SO₄, and the N concentration was consistent with the IRRI rice nutrient solution (Yoshida et al., 1972). As soil-solution NH₄⁺ concentrations in paddy soil can easily fall into the 2–20 mM range (Glass et al., 2002), the high-NH₄⁺ concentrations were set to 2, 5, 10, 15 mM, provided as 1, 2.5, 5, 7.5 mM (NH₄)₂SO₄, 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-o-old seedlings were used for measurements.

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

After treatment, 32-o-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 H₂SO₄-H₂O₂ 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 80 μm long, were collected from 32-o-old seedlings in the three cultivars to evaluate aerenchyma formation by scanning electron 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 Borkhousen, 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$_4^+$ 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 (0.5 mM NH$_4$Cl, pH = 5.5) for 30 min, they 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$_4$Cl, 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
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$_4$NO$_3$) 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 ($K_m$) 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
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
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 \( \text{NH}_4^+ \) into \( \text{NO}_3^- \) 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 \( \text{NO}_3^- \) then raise the thresholds of toxic \( \text{NH}_4^+ \) concentration and reduce \( \text{NH}_4^+ \) toxicity, because \( \text{NO}_3^- \) uptake is associated with alkalinization of the root medium and stimulation of cation uptake, counteracting some of the effects of \( \text{NH}_4^+ \) (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 \( \text{NH}_4^+ \) uptake

The capacity of dealing with \( \text{NH}_4^+ \) 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 \( \text{NH}_4^+ \) uptake (Britto and Kronzucker, 2006; Lea and Azevedo, 2006). Using an \( ^{15}\text{N} (^{15}\text{NH}_4^+) \) labeling method, it was observed that maximum uptake rates (\( V_{\text{max}} \)) and Michaelis constants (\( K_m \)) in the hybrid super rice lines YY and JY were significantly higher than those in XS. \( V_{\text{max}} \) is typically reflective of the number of ion transporters present in cell membranes, while \( K_m \) 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 \( \text{NH}_4^+ \) uptake in the super rice cultivars can therefore reasonably be attributed to an increased number of \( \text{NH}_4^+ \) carriers. Roots of YY and JY had a higher capacity for \( \text{NH}_4^+ \) uptake.
uptake, but XS displayed a higher affinity for NH₄⁺. 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 NH₄⁺ and this correlates with reduced NH₄⁺ efflux from roots

High concentrations of NH₄⁺ 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 NH₄⁺ toxicity than commonly used varieties. NH₄⁺ influx is mediated by energetically active high-affinity transport systems (HATS) in the plasma membranes of root cells at low NH₄⁺ concentrations, and by high-capacity, energetically passive, low-affinity transport systems (LATS) under high NH₄⁺ 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 Wieren 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 NH₄⁺ inhibition of primary root growth, and inhibition of cell elongation was associated with NH₄⁺ efflux in the root elongation zone, the elongation zone was more sensitive to NH₄⁺ stress than maturation zone. Chen et al. (2013) found that NUE in rice was associated with NH₄⁺ cycling and tissue accumulation in the elongation zone of the root. Based on these findings, we measured NH₄⁺ fluxes at the surface of the elongation zone of roots in the three selected rice cultivars. Under moderate NH₄⁺ levels, significantly larger NH₄⁺ fluxes were detected at the elongation zones in YY and JY, while NH₄⁺ effluxes were detected in XS. At high NH₄⁺ concentrations, the direction of NH₄⁺ flux in YY and JY, however, switched, and NH₄⁺ 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 NH₄⁺ 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 NH₄⁺ in the seedling stage and that this is associated with improved regulation of futile NH₄⁺ 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 NH₄⁺ 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.

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 NH₄⁺. Values shown are the means ± SD of three replicates. Different letters represent significant differences (Duncan’s test, at P < 0.05).

Fig. 7. ¹⁵NH₄⁺ 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.
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.

Funding

This work was supported by the National Key Research and Development Program of China (Grant No. 2018YFD0800204, 2017YFD0200103) and National Natural Science Foundation of China (31572205).

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.

Acknowledgements

We thank Deling Sun (Center for Soil and Environment Analysis and Test, Institute of Soil Science, Chinese Academy of Sciences), Jing Yang (Advanced Analysis and Testing Center, Nanjing Forestry University), Yunqi Liu (Xuyue Sci. & Tech. Co., Ltd) for kindly providing help in root testing, and Guangjie Li (State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences) for providing constructive suggestions on the experiment.

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


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Table 2

Kinetics parameters of 15NH4+ uptake in XS, YY, and JY seedlings (means ± SD, n = 3). Vmax and Km were determined by a non-linear curve-fitting model (Michaelis–Menten kinetics). DW: dry weight.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Kinetic equation</th>
<th>R²</th>
<th>Km (μmol/L)</th>
<th>Vmax (μmol g⁻¹ DW h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common variety</td>
<td>XS v = 22.23*[C]/(0.09625 + [C])</td>
<td>0.9428</td>
<td>96.25 ± 20.04b</td>
<td>22.23 ± 0.94b</td>
</tr>
<tr>
<td>Hybrid super rice</td>
<td>YY v = 37.68*[C]/(0.2148 + [C])</td>
<td>0.9865</td>
<td>214.8 ± 21.18a</td>
<td>37.68 ± 0.96a</td>
</tr>
<tr>
<td></td>
<td>JY v = 35.59*[C]/(0.1834 + [C])</td>
<td>0.9611</td>
<td>183.4 ± 31.09a</td>
<td>35.59 ± 1.49a</td>
</tr>
</tbody>
</table>

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


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