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Ammonium enhances the tolerance of rice seedlings (*Oryza sativa* L.) to drought condition

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ABSTRACT

In order to study the effects of different nitrogen (N) forms on drought tolerance of rice seedlings, both hydroponic and pot experiments were conducted in green house. In hydroponic experiments, water stress was simulated by treatment with polyethylene glycol (PEG, 10% in w/v, MW6000); in pot experiments, rice seedlings were cultivated under non-flooded conditions. The results showed that: (1) under water stress conditions, the decrease in plant growth and photosynthesis under ammonium supply was less than under nitrate supply; (2) under non-flooded cultivation, the biomass and photosynthesis in rice plants supplied with ammonium and ammonium + dicyandiamide (DCD, a nitrification inhibitor) were higher than those in nitrate fertilization; (3) in hydroponic experiments, water uptake of rice seedlings under ammonium nutrition was higher than under nitrate nutrition. It is concluded that, ammonium nutrition can enhance the tolerance of rice plants to water stress.

1. Introduction

In rice production more than 80% of the water irrigated to a paddy field is lost through evaporation and leakage, only less than 20% is transpired by the rice itself. Water use efficiency in the rice production system is extremely low, to produce 1 kg rice grain about 2 m^3 water is needed (Belder et al., 2005; Liu et al., 2005), which is 2–3 times more water than other cereal crops (Tuong et al., 2005). This wasteful utilization of fresh water in traditional rice cultivation is causing more and more concerns. To develop a water-saving irrigation system in rice production, some field experiments were conducted to examine rice growth under non-flooded conditions (water content was maintained between 70 and 90% of soil saturated water content) in China (Tao et al., 2006; Fan et al., 2005; Liu et al., 2005), however in this system the growth of the rice seedling is frequently inhibited by water stress.

Rice is very sensitive to water stress; Granier and Tardieu (1999) showed that water deficiency reduced leaf relative expansion and cell division rates and ultimately lead to decreased leaf area. Water deficiency also resulted in reduced leaf water potential, leaf rolling, decreased stomatal (g_s) and mesophyll (g_m) conductance to CO_2 (O'Toole and Cruz, 1980; Lauteri et al., 1997). The reduced g_s and g_m resulted in a decrease in intercellular CO_2 concentration (C_i) and/or in chloroplastic CO_2 concentration (C_c), and ultimately resulted in decreased photosynthetic capacity and grain yield (Kaiser, 1987; Lal et al., 1996).

One major difference between flooded and non-flooded fields was the available nitrogen (N) form changes, i.e. from ammonium under water logging to nitrate and/or the mixture of ammonium and nitrate in aerobic conditions (reviewed by Guo et al., 2007a). Numerous studies have demonstrated that different N forms significantly influenced plant growth, but contrasting results were observed depending on the plant species used. Some upland plants such as maize, wheat, tobacco, bean and sugar beet, preferred nitrate to ammonium nutrition (Guo et al., 2002; Walch-Liu et al., 2000; Raab and Terry, 1994). These plants would suffer ammonium toxicity when supplied with high ammonium in the root medium as the sole N source. Having a higher ammonium assimilation capacity than other plant species, rice plants could avoid ammonium toxicity and exhibited a preference for ammonium nutrition (Britto et al., 2001; Guo et al., 2007a). Both changes in N supply form and water status under non-flooded conditions, were shown to influence the growth of rice plants (Guo et al., 2007c, 2008). This integrated effect on rice growth was highly dependent on both photosynthetic characteristics and water relations (Makino et al., 1985; Long et al., 2006; Guo et al., 2007a).

In previous studies, it was evidenced that, under drought stress, rice seedlings grow better in ammonium nutrition (Guo et al., 2007a,c, 2008). However, the question still remains open, whether these ammonium effects could maintain under drought stress in soil culture system. In the present study, it was hypothesized that maintaining a certain proportion of ammonium in soil under drought stress or non-flooded field could affect the growth of rice

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plants. To identify this physiological response, both hydroponic (water stress was simulated by adding PEG, 6000) and pot experiments (non-flooded soil) were conducted in the green house, and we investigated the relationship between the form of N supply on rice growth and photosynthetic characteristics.

2. Materials and methods

2.1. Plant material and growth conditions

After germination on moist filter paper, rice (*Oryza sativa* L.) seeds (cultivars, *cv.* "Shanyou 63" hybrid *indica*. China) were transferred to a 2.0 mol m^{-3} $CaSO_4$ solution for germination. Three days later, seedlings were transferred to a 1/4-strength mixture of NO_3^- and NH_4^+ containing nutrient solution (for composition, see below). After a further three days, seedlings were transferred to a half-strength mixture of the same nutrient solution. Five days later the seedlings were supplied with full-strength mixture of NO_3^- and NH_4^+ containing nutrient solution for one week. Seedlings were supplied with the nutrient solutions containing either NH_4^+ or NO_3^- for the N form treatments. All the treatments had four replicates arranged in a completely random design to avoid edge effects in the greenhouse.

Composition of the nutrient solution for hydroponic culture was as follows: macronutrients: (mol m^{-3}): 2.85 N as $(NH_4)_2SO_4$ or $Ca(NO_3)_2$; 1.02 K as K_2SO_4 and KH_2PO_4; 0.32 P as KH_2PO_4, 1.65 Mg as $MgSO_4$, micronutrients (mmol m^{-3}): 35.8 Fe as Fe-EDTA; 9.10 Mn as $MnSO_4$; 0.15 Zn as $ZnSO_4$; 0.16 Cu as $CuSO_4$; 18.5 B as H_3BO_3; 0.52 Mo as $(NH_4)_6Mo_7O_{24}$; 0.1 Si as Na_2SiO_4. In ammonium-containing nutrient solution, Ca^{2+} was supplied as $CaCl_2$ (1.43 mol m^{-3}). The nitrification inhibitor (DCD) was added to every pot. Water stress was simulated by adding 10% PEG (6000) to the nutrient solutions (−0.15 MPa, Michel and Kaulmann, 1973). The pH of the nutrient solution was maintained at 5.50 ± 0.05 by adding 1 mol L^{-1} HCl or 1 mol L^{-1} NaOH to the solutions every day. The nutrient solutions were changed every two days. Seven days after the simulated water stress was applied, gas exchange, water uptake and xylem sap measurements were taken. One day later, seedlings were harvested and separated into root, stem and leaf fractions. All samples were oven-dried at 105 °C first for 30 min, and then 70 °C to constant weight.

In order to examine whether N form could influence drought resistance of rice plants in non-flooded soil, a synchronous pot experiment was conducted, rice seedlings with 2–3 visible leaves ("Yangdao 6" China, a less drought resistance variety, see Guo et al., 2008) were transplanted to pots with 3 kg soil per pot. Total N of the soil was 1.29 g kg^{-1}, available P_2O_5 was 55 mg kg^{-1}, available K_2O was 18 mg kg^{-1} and pH was 7.34. Different nitrogenous fertilizer forms $(NH_4)_2SO_4$, $Ca(NO_3)_2$ and $(NH_4)_2SO_4$ + DCD, together with P as KH_2PO_4, and K as KCl were applied to the soil before transplanting. Soil moisture was maintained to roughly 85% (by weight) by irrigation twice a day. Rice plants were measured and sampled at booting stage.

Rice plants were grown in the greenhouse at 25/18 °C day/night temperatures. Light was supplied with SON-T AGRO 400W bulbs, keeping light intensity in a 14 h photoperiod at a minimum of 1000 μmol photons m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at the leaf-level.

2.2. Gas exchange measurements

The light saturated photosynthetic rate of new fully expanded leaves was measured from 9:00 to 15:00 with a Li-Cor 6400-02B portable photosynthesis open-system. Leaf temperature during measurements was maintained at 28 °C, with photosynthetic photon flux intensity (PPFD) of 1500 μmol photons m^{-2} s^{-1}. Ambient CO_2 concentration in the cuvette (C_{a-c}) was adjusted to atmospheric CO_2 concentration (C_a) (380 μmol CO_2 mol^{-1}), and relative humidity was maintained at 50.19 ± 1.93%. Data were recorded after equilibration to a steady state (approximately 10 min). The measured leaves were labeled and leaf areas were calculated.

One day later, A/C_i response curves were measured. Leaf temperature, PPFD and relative humidity were maintained during measurements as mentioned above. Ten minutes prior to initiating measurements, leaves were placed in the cuvette at a PPFD of 1500 μmol photons m^{-2} s^{-1}, C_{a-c} was maintained to 400 μmol CO_2 mol^{-1} with a CO_2 mixer. Ten minutes later, C_{a-c} was controlled across the series of 200, 150, 100, 50 μmol CO_2 mol^{-1} and data were recorded after equilibration to a steady state. Carboxylation efficiency (CE) was calculated as the initial slope of A/C_i response curves.

2.3. The rate of water uptake and xylem sap flow measurements

Seven days after simulated water stress, water uptake rate was measured by weighing between 9:00 and 11:00 on a sunny day. After the measurement of water uptake, xylem sap was collected according to a previously described method (Soejima et al., 1992). Plants were de-topped about 2 cm above the interface of shoot and root at 8:00 PM. Absorbent cotton was placed on the top of each de-topped xylem and covered with a polyethylene bag. After 24 h the cotton and root were collected, the exudation rate was estimated from the change weight of the cotton and it was expressed in terms of the root fresh weight.

All data were analyzed using the statistical software package SAS 9.0 and Microsoft Excel 2003, with analysis of variance for treatments and five replications. Significant differences ($P < 5\%$) between treatments are indicated by different letters.

3. Results

Under non-water stress conditions, there were no significant differences in root and shoot dry mass between plants grown with the two forms of N supply, but total leaf area was higher in nitrate than in ammonium grown plants (Table 1). When compared to non-water stress conditions, under water stress, shoot dry mass decreased by 25% and 43%, and leaf area was reduced by 23% and 52% under ammonium and nitrate nutrition, respectively (Table 1).

Under non-water stress conditions, there were no significant differences in net photosynthetic rate (Pn), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and carboxylation efficiency (CE) between plants supplied with both N forms (Table 2). When compared with non-water stress conditions, significant reductions in Pn, g_s, transpiration rate (E), and CE were observed under nitrate nutrition under water stress, whereas no significant change was observed in any photosynthetic parameter under ammonium nutrition (Table 2). Under water stress conditions, carboxylation efficiency (CE) was higher in plants

Table 1
Effects of different N forms and water stress on dry mass of rice seedlings (*cv.* "Shanyou 63" hybrid *indica*. China). Plants were supplied with sole ammonium (NH_4^+ as AN) or sole nitrate (NO_3^- as NN) under non-water stress or water stress simulated by adding 10% (w/v) PEG 6000 (NH_4^+ + PEG as ANP, NO_3^- + PEG as NNP). Data were means ± SE of five replications, and significant differences ($P < 5\%$) among different treatments were indicated by different letters.

Treatments	Root (g $plant^{-1}$)	Shoot (g $plant^{-1}$)	Leaf area (cm^2 $plant^{-1}$)	Number of tillers
AN	0.20 ± 0.02 ab	0.69 ± 0.07 a	70.3 ± 10.8 b	4.60 ± 0.55 a
ANP	0.20 ± 0.05 ab	0.52 ± 0.08 b	54.9 ± 7.0 c	4.40 ± 0.55 ab
NN	0.21 ± 0.01 a	0.71 ± 0.12 a	88.3 ± 15.6 a	3.60 ± 0.89 bc
NNP	0.17 ± 0.03 b	0.41 ± 0.05 c	42.5 ± 0.9 d	3.40 ± 0.55 c