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The enhanced drought tolerance of rice plants under ammonium is related to aquaporin (AQP)☆

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ABSTRACT

Previously, we demonstrated that drought resistance in rice seedlings was increased by ammonium (NH_4^+) treatment, but not by nitrate (NO_3^-) treatment, and that the change was associated with root development. To study the effects of different forms of nitrogen on water uptake and root growth under drought conditions, we subjected two rice cultivars (cv. 'Shanyou 63' hybrid *indica* and cv. 'Yangdao 6' *indica*, China) to polyethylene glycol-induced drought stress in a glasshouse using hydroponic culture. Under drought conditions, NH_4^+ significantly stimulated root growth compared to NO_3^-, as indicated by the root length, surface area, volume, and numbers of lateral roots and root tips. Drought stress decreased the root elongation rate in both cultivars when they were supplied with NO_3^-, while the rate was unaffected in the presence of NH_4^+. Drought stress significantly increased root protoplast water permeability, root hydraulic conductivity, and the expression of root aquaporin (AQP) plasma intrinsic protein (PIP) genes in rice plants supplied with NH_4^+; these changes were not observed in plants supplied with NO_3^-. Additionally, ethylene, which is involved in the regulation of root growth, accumulated in rice roots supplied with NO_3^- under conditions of drought stress. We conclude that the increase in AQP expression and/or activity enhanced the root water uptake ability and the drought tolerance of rice plants supplied with NH_4^+.

1. Introduction

Drought stress is a major abiotic stress factor limiting plant growth and crop productivity [1]. Understanding the genetic and biochemical processes that regulate drought tolerance is a vital area of research in plant biology. Previous studies have focused on the relationship between nutritional status and drought resistance and the integrated effects of nutrients and water status on photosynthesis and water relations [2,3]. Previously, we demonstrated that ammonium (NH_4^+) enhanced the drought tolerance of rice seedlings [4–7]. In addition to rice, the effects of NH_4^+ and nitrate (NO_3^-) have been studied intensively in others plant species, including maize, wheat, tobacco, bean and sugar beet [8–11]. NH_4^+ and NO_3^- have different impacts on physiological and biochemical processes in higher plants, including plant growth, photosynthesis, photorespiration and water relations [12]. Guo et al. [13] showed that French bean plants exhibited higher rates of water uptake when supplied with NO_3^- than with NH_4^+. In short-term experiments conducted on tobacco, no difference in water uptake rate was found between plants supplied with two different forms of nitrogen [10]. However, the effects of different nitrogen forms on plant water uptake might depend on the ontogenetic stage and the plant species [12].

We previously demonstrated that rice seedlings supplied with NH_4^+ adapted to drought stress through enhanced root growth with increased numbers of root tips and a larger root surface area, which

Abbreviations: AQP, Aquaporin (water channels protein); MIP, major intrinsic membrane proteins; PIP, plasma membrane intrinsic protein; AN, ammonium; NN, nitrate; ANP, ammonium plus 10% (w/v) PEG6000; NNP, nitrate plus 10% (w/v) PEG6000.

☆ This study was conducted in greenhouse in Nanjing Agricultural University, Tongwei Road 6, Nanjing, Jiangsu 210095, China.

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increased water uptake [5]. Yang et al. [7] reported that drought-induced root aerenchyma formation restricted water uptake in rice seedlings supplied with NO_3^-. These results indicated that the water-uptake ability of rice seedling roots under drought stress conditions was higher with NH_4^+ nutrition than with NO_3^- nutrition due to effects on root growth.

Extensive root systems are vital to sustain sufficient water absorption in plants subjected to drought stress [14]. The mechanisms involved in root growth maintenance under water deficits were reviewed by Sharp et al. [1] and include changes in growth zone dimensions, turgor maintenance by osmotic adjustment, enhanced cell wall loosening and changes in abscisic acid (ABA) accumulation. However, how root growth is regulated by different nitrogen forms under drought stress remains unknown. Root growth results from cell division and expansion, which requires continuous water uptake to maintain turgor pressure; that pressure is controlled by a gradient in water potential across cellular membranes established through the accumulation of solutes [15]. The high water permeability found in most biological membranes, including the plasma membrane, tonoplast and chloroplastic envelope, results from the presence of aquaporin (AQP) water channels. AQPs facilitate water movement across membranes and numerous AQPs have been identified in plants [16].

AQPs are major intrinsic membrane proteins (MIPs), which are classified into five subfamilies: plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin 26-like intrinsic proteins (NIPs), small intrinsic proteins (SIPs) and X intrinsic proteins (XIPs) [17]. Among these, PIPs were shown to have clear water transport functions in both *in vivo* and *in vitro* experiments [18–20]. *PIP* gene expression was reported to be down- or up-regulated under unfavorable conditions such as salt, drought or cold stress [21–24], suggesting the involvement of *PIP* genes in the maintenance of plant water balance. In addition to stress responses, AQPs also play a role in the effects of nitrogen forms on plant water uptake as demonstrated by higher *PIP* gene expression and an associated increase in the rate of water uptake in French bean supplied with NO_3^- [13]. Different levels of drought resistance in rice seedlings provided with different nitrogen forms might be due to the regulation of root AQPs with consequent effects on root water status and growth.

In this study, we examined the effects of drought stress and different nitrogen forms on root growth, AQP gene expression in roots, and protoplast water permeability. We discuss the effect of root AQP expression on the drought tolerance of rice plants, as well as the interaction of AQPs with different forms of nitrogen and how it affects root growth under conditions of drought stress.

2. Materials and methods

2.1. Plant material and growth conditions

Two rice cultivars were selected, with different drought tolerance, including *Oryza sativa* L., cv. 'Shanyou 63' hybrid *indica* China, drought tolerance and 'Yangdao 6' *indica* China, drought sensitive. Rice seeds were disinfected in 10% H_2O_2 (W/W) for 30 min and then germinated in saturated $CaSO_4$ solution for 2 days. After the seedlings had developed an average of 2.5 visible leaves, they were transplanted to 7-L buckets containing a quarter-strength mixture of NH_4^+ and NO_3^- (ANN) nutrient solution (for composition, see below). After 3 days, the rice seedlings were transferred to half-strength ANN for 5 days and then supplied with full-strength ANN for 1 week after which the seedlings were supplied with either NH_4^+ (AN) or NO_3^- (NN) nutrient solution. After an additional week, the seedlings were subjected to simulated drought stress by the addition of 10% polyethylene glycol (PEG, 10% w/v, MW 6000) to the nutrient solutions (−0.15 MPa). Four treatments were applied: AN, NN, NH_4^+ plus 10% PEG 6000 (ANP) and NO_3^- plus 10% PEG 6000 (NNP). The nutrient compositions of the solutions were as follows. Macronutrients included 40 mg L^{-1} N as $(NH_4)_2SO_4$ or $Ca(NO_3)_2$, 10 mg L^{-1} phosphorus (P) as KH_2PO_4, 40 mg L^{-1} potassium (K) as K_2SO_4 and KH_2PO_4 and 40 mg L^{-1} magnesium (Mg) as $MgSO_4$. Micronutrients included 2.0 mg L^{-1} iron (Fe) as Fe–EDTA, 0.5 mg L^{-1} manganese (Mn) as $MnCl_2{\cdot}4H_2O$, 0.05 mg L^{-1} molybdenum (Mo) as $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.2 mg L^{-1} boron (B) as H_3BO_3, 0.01 mg L^{-1} zinc (Zn) as $ZnSO_4{\cdot}7H_2O$, 0.01 mg L^{-1} copper (Cu) as $CuSO_4{\cdot}5H_2O$, and 2.8 mg L^{-1} silicon (Si) as $Na_2SiO_3{\cdot}9H_2O$. The Ca content in treatment A was compensated for by the addition of $CaCl_2$. The nitrification inhibitor dicyandiamide (DCD) was added to each nutrient solution to maintain the specified condition. Nutrient solutions were changed at 3-day intervals and the pH was adjusted daily to 5.50 ± 0.05 with 0.1 mol L^{-1} HCl or 0.1 mol L^{-1} NaOH.

All treatments were replicated five times using a completely random design. The various treatments were placed randomly in the glasshouse to avoid edge effects. The temperature in the glasshouse was maintained at 30 °C during the day and 18 °C at night. Light was supplied by SON-T AGRO 400 W bulbs; the light intensity was maintained at a minimum of 1000 μmol photons m^{-2} s^{-1} (photosynthetically active radiation) at the leaf level using a 14-h photoperiod.

2.2. Root morphological characteristics and the root elongation rate

After 8 days of stress treatment, rice roots were immersed in water and fine roots were separated gently using a toothpick to avoid injury before imaging. Morphological measurements were taken using an LA1600 scanner (Regent Instruments, Sainte-Foy-Sillery-Cap-Rouge, QC, Canada) and WinRHIZO2008a software (Regent Instruments).

For root elongation rate measurements, five adventitious roots from each treatment were marked with active carbon. An initial root length measurement was made and the roots were measured again 1 day later. The root elongation rate was calculated based on the measured change in length.

2.3. Frozen sections for determining root cell size

After 8 days of stress treatment, roots were cut into approximately 0.5-cm segments and embedded in embedding medium at approximately −14 °C. After 30 min, the frozen roots were sectioned and the root cells were observed under a microscope. Root cell size was calculated from 20 to 30 representative cells (the third or fourth layer of cortical cells, 25–30 mm from the root tip).

2.4. Root tissue RNA extraction, cDNA synthesis and quantitative reverse transcription (qRT)-PCR

After 1 day of simulated drought stress treatment, root samples were harvested, frozen immediately in liquid nitrogen, and stored at −70 °C for subsequent RNA isolation. Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. cDNA was synthesized using Revert Aid Reverse Transcriptase (Fermentas, Waltham, MA, USA) according to the manufacturer's instructions. The expression levels of nine rice plasma membrane intrinsic protein (*OsPIP*) genes were analyzed by qRT-PCR using an ABI 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) and the products were labeled using the SYBR Green Master Mix (SYBR Premix Ex *Taq* II; TaKaRa) according to the manufacturer's instructions. The