



Research article

Ammonium uptake and metabolism alleviate PEG-induced water stress in rice seedlings



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ABSTRACT

Ammonium (NH_4^+) can enhance the water stress induced drought tolerance of rice seedlings in comparison to nitrate (NO_3^-) nutrition. To investigate the mechanism involved in nitrogen (N) uptake, N metabolism and transcript abundance of associated genes, a hydroponic experiment was conducted in which different N sources were supplied to seedlings growing under water stress. Compared to nitrate, ammonium prevented water stress-induced biomass, leaf SPAD and photosynthesis reduction to a significantly larger extent. Water stress significantly increased root nitrate reductase (NR) and nitrite reductase (NiR) activities, but decreased leaf NiR and glutamate synthetase (GS) activities under NO_3^- supply, causing lower nitrate content in roots and higher in leaves. In contrast, under NH_4^+ supply root GS and glutamine oxoglutarate aminotransferase (GOGAT) activities were significantly decreased under water stress, but remained higher in leaves, compared to NO_3^- treatment, which was beneficial for the transport and assimilation of ammonium in leaves. ^{15}N tracing assays demonstrated that rice ^{15}N uptake rate and accumulation were significant reduced under water stress, but were higher in plants supplied with NH_4^+ than with NO_3^- . Therefore, the formers showed higher leaf soluble sugar, proline and amino acids contents, and in turn, associated with a higher photosynthesis rate and biomass accumulation. Most genes related to NO_3^- uptake and reduction in roots and leaves were down-regulated; however, two ammonium transporter genes closely related to NH_4^+ uptake (*AMT1;2* and *AMT1;3*) were up-regulated in response to water stress. Overall, our findings suggest that ammonium supply alleviated water stress in rice seedlings, mainly by increasing root NH_4^+ uptake and leaf N metabolism.

1. Introduction

Although rice is traditionally cultivated under water logged conditions, water stress increasingly becomes a most severe limitation to crop productivity in major rice production areas around the world due to wasteful water use and seasonal water shortage in some semiarid ecosystems (Tuong and Bouman, 2003; Zwart and Bastiaanssen, 2004). Low water availability induces various physiological, biochemical and molecular responses in plants, as indicated by significant decline in leaf area, chlorophyll content and photosynthesis, as well as altered enzyme activity and gene expression, all of which eventually limit plant growth

(Bogeat-Triboulot et al., 2007; Lei et al., 2006; Monclus et al., 2006). Since, soil available nitrogen (N) is mainly absorbed by roots and assimilated in both shoot and root, achieving a high crop performance requires a clear understanding of N uptake and subsequent metabolism in rice, in response to water stress.

Additionally, the negative effects of soil water stress on plant N metabolism, such as reduction in N-metabolism enzymes activities and synthesis of nitrogenous compounds, have been widely demonstrated (Pinheiro and Chaves, 2011; Xu and Zhou, 2006). However, crops supplied with greater amounts of N have been shown to withstand drought better by enhancing developmental plasticity of the root

Abbreviations: NR, nitrate reductase; NiR, nitrite reductase; GS, glutamate synthetase; GOGAT, glutamine oxoglutarate aminotransferase; P_n , photosynthesis; C_i , leaf CO_2 concentration; g_s , stomatal conductance; WUE, water use efficiency; RT-PCR, reverse-transcriptase polymerase chain reaction

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system (Tran and Yamauchi, 2014; Huang et al., 2018a), and increasing photosynthetic machinery and antioxidant capacity (Zhong et al., 2017). Synthesis of nitrogenous compounds, e.g. amino acid and soluble protein, help in maintaining osmotic balance in defense against plant water stress (Ashraf and Foolad, 2007; Cai et al., 2008). On the other hand, N sources also have a strong influence on plant growth through the regulation of photosynthesis and carbon/nitrogen metabolism. As the two primary inorganic N sources available for plants in soils, ammonium (NH_4^+) and nitrate (NO_3^-) differ greatly with respect to their impact on the negative effects of water stress on plant development (Guo et al., 2007a,b). Furthermore, the positive effects of NH_4^+ supply on plant growth, physiological performance and photosynthesis in rice seedlings have been sufficiently demonstrated. Such is the case of the significant differences observed in root morphology, leaf SPAD, gas exchange parameters (e.g. stomatal conductance (g_s) and intercellular CO_2 concentration [C_i]), chlorophyll and Rubisco contents or Rubisco activity (Guo et al., 2007a,b). Chloroplast upsizing in plant leaves supplied with NH_4^+ under water stress was shown to enhance CO_2 supply to Rubisco via increased leaf mesophyll conductance (g_m) and g_s ; thus, improving leaf photosynthetic rate (Li et al., 2012). On the other hand, NH_4^+ -supplied rice seedlings showed relatively higher water uptake rates and xylem sap flow rates than NO_3^- -supplied plants under water stress conditions by enhanced root aerenchyma formation and induced higher expression of root aquaporin (AQP) and plasma intrinsic protein (PIP) genes (Ding et al., 2015; Yang et al., 2012).

After uptake, NH_4^+ can be converted into amino acids directly, whereas NO_3^- needs to be reduced to NH_4^+ by nitrate reductase (NR) and nitrite reductase (NiR), before incorporation into amino acids (Luo et al., 2013; Rennenberg et al., 2010). Nunes-Nesi et al. (2010) demonstrated that most N assimilated in plants is invested in the photosynthetic machinery. In this process, the reduction of photosynthetic rate induced by water stress was accompanied by the decrease in the activities of N metabolism enzymes, such as NR, glutamine synthetase (GS), glutamine oxoglutarate aminotransferase (GOGAT), and glutamine dehydrogenase (GDH) (Foyer et al., 1998; Sanchez-Rodriguez et al., 2011). Previously, water stress has been reported to significantly reduce NH_4^+ and NO_3^- uptake in maize (Buljovic and Engels, 2001), and *Populus simonii* (Meng et al., 2016). At the molecular level, water stress down-regulated genes related to NO_3^- uptake and reduction, and N metabolism, but up-regulated NH_4^+ uptake genes (*AMT1;2*, *AMT1;6*, *AMT4;2* and *AMT4;3*) at high N levels (Meng et al., 2016; Huang et al., 2018a; b). Due to the low energy requirement, NH_4^+ assimilation is advantageous for plant growth as compared with NO_3^- (Salsac et al., 1987). These studies indicate that the N uptake and metabolism play an important role in the acclimation of plants to water stress condition. To the best of our knowledge, although ammonium-induced resistance of rice to water stress has been widely demonstrated, the effect of water stress on NH_4^+ and NO_3^- uptake, metabolism, and transcriptional regulation of their transporters has not been thoroughly studied.

In this study, the effects of water stress on N uptake and metabolism, activities of some of the main enzymes involved in N metabolism, as well as gene expression involved in N uptake in NH_4^+ -/ NO_3^- -supplied rice seedlings, were investigated in a hydroponic experiment. We hypothesized that (1) N uptake and accumulation in rice seedlings is inhibited by water stress, but that negative effects are significantly alleviated by NH_4^+ supply; (2) N transporter genes and subsequent N metabolism under NH_4^+ supply jointly enhance drought resistance of rice seedlings.

2. Materials and methods

2.1. Rice seedling cultivation

Rice (*Oryza sativa* L. ‘Zhongzheyu No. 1’ hybrid indica) seedlings were grown hydroponically in a greenhouse. After germination, seeds were transferred to a 0.5 mmol L⁻¹ CaCl_2 (pH 5.5) solution for growth.

Three days later, seedlings were transferred to 1.5 L black plastic pots containing a mixture of NO_3^- and NH_4^+ nutrient solution with the following composition: NH_4NO_3 (0.5 mM), $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (0.18 mM), KCl (0.18 mM), CaCl_2 (0.36 mM), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.6 mM), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (9 μM), $\text{Na}_2\text{MoO}_4 \cdot 4\text{H}_2\text{O}$ (0.1 μM), H_3BO_3 (10 μM), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0.7 μM), CuSO_4 (0.3 μM), and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ -EDTA (20 μM). All the experiments were performed in the controlled environment of a growth room, which was maintained under the following conditions: 14 h/10 h (light/dark) photoperiod, 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity during the illuminated phase, 28 °C or 23 °C during day or night, respectively, and 60% relative humidity.

After seven days, seedlings of similar size were further cultivated under one of the following treatments: NO_3^- , $\text{NO}_3^- + \text{PEG-6000}$, NH_4^+ or $\text{NH}_4^+ + \text{PEG-6000}$. For NH_4^+ and NO_3^- treatments, 1 mM NH_4^+ and 1 mM NO_3^- were added to the nutrient solution, respectively. Water stress was induced by adding 10% PEG-6000 to the solution, and the PEG-free solution was set as the control for each N treatment. Other nutrients were also provided as described above; pH of the nutrient solution was buffered with 5 mM 2-(4-Morpholino)ethanesulfonic acid (MES) to 5.5, and the nutrient solution was renewed every three days. Treatments under hydroponic cultivation lasted for three weeks before harvest.

2.2. Root ¹⁵N uptake rate

After cultivation for three weeks, plant ¹⁵N uptake was determined according to the method previously described (Cao et al., 2017): Firstly, seedlings were treated with deionized water for 4 h to develop a “starvation condition”. Thereafter, N source was replaced in the nutrient solution with ¹⁵N-labeled ¹⁵ NO_3^- and ¹⁵ NH_4^- substrates (50 atom%). In addition to N, nutrient solution also contained the macro and micronutrients described above. After 6 h of incubation, the root system was cut off, washed with 50 mM CaCl_2 , and rinsed three times with deionized water. Rice shoot and root samples were immediately freeze-dried (Labconco FreezenSystem, USA), then ground to a fine powder with a ball mill (RetschMM301, Germany). ¹⁵N enrichment in the freeze-dried root and shoot samples, and N content were determined using a Tracer MAT-271 (Finnigan MAT, USA).

2.3. Gas exchange measurement

Measurement of leaf gas exchange parameters was conducted on sunny days at ambient CO_2 concentration (C_a , about 390 mmol mol⁻¹) using a portable photosynthesis system (Li-6400XT, Li-Cor Co. Ltd., USA). Fully expanded leaves were measured from 09:00 to 12:00; leaf temperature was maintained at 28 °C, with a photosynthetic photon flux density (PPFD) of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 60%–70% relative humidity. Gas exchange parameters calculated included: net photosynthetic rate (P_n), intercellular CO_2 concentration (C_i), and stomatal conductance (g_s), and transpiration rate (T_r). Water use efficiency (WUE) was calculated as the ratio of net photosynthesis (A) to T_r ($\text{WUE} = A/T_r$). Leaf water potential was determined with a WP4C Dewpoint Potential Meter (Decagon, USA). Here, the fully expanded leaves were sampled, cut into 0.4 cm length pieces, and then measured from 09:00 to 13:00.

Rice seedlings were harvested and divided into leaves, shoots and roots. Thereafter, one portion of the samples was oven-dried, first at 105 °C for 30 min and then at 70 °C until constant weight. The other portion of the samples were immediately frozen in liquid N, ground into a fine powder, and stored at –80 °C wrapped with tinfoil.

2.4. NO_3^- , NH_4^+ , proline and total amino acids

Leaf proline content was estimated using the methods described previously with some modifications (Bates et al., 1973): leaf and root samples (0.5 g) were homogenized in 7.5 ml of 3% sulfosalicylic acid

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