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Research article

Glycine increases cold tolerance in rice via the regulation of N uptake, physiological characteristics, and photosynthesis

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ABSTRACT

To investigate the response of rice growth and photosynthesis to different nitrogen (N) sources under cold stress, hydroponic cultivation of rice was done in greenhouse, with glycine, ammonium, and nitrate as the sole N sources. The results demonstrate that exposure to low temperature reduced the rice biomass and leaf chlorophyll content, but their values in the glycine-treated plants were significantly higher than in the ammonium- and nitrate-treated plants. This might be attributed to the higher N uptake rate and root area and activity in the glycine-treated plants. The glycine-treated plants also maintained high contents of soluble proteins, soluble sugars, and proline as well as enhanced antioxidant enzyme activities to protect themselves against chilling injury. Under cold stress, reduced stomatal conductance (g_s) and effective quantum efficiency of PSII (Φ_{PSII}) significantly inhibited the leaf photosynthesis; however, glycine treatment alleviated these effects compared to the ammonium and nitrate treatments. The high non-photochemical quenching (qN) and excess energy dissipative energy (E_x) in the glycine-treated plants were beneficial for the release of extra energy, thereby, strengthening their photochemical efficiency. We, therefore, conclude that the strengthened cold tolerance of glycine-treated rice plants was closely associated with the higher accumulation of dry matter and photosynthesis through the up-regulation of N-uptake, and increase in the content of osmoprotectants, activities of the antioxidant defense enzymes, and photochemical efficiency. The results of the present study provide new ideas for improving the plant tolerance to extreme temperatures by nutrient resource management in the cold regions.

1. Introduction

Plant species have evolved to function under optimal environmental conditions, for example, of light and temperature; deviations in these conditions can result in the reduction of plant growth, inhibition of photosynthesis, imbalanced ion uptake, and oxidative stress (Sims et al., 2012; Genisel et al., 2013). Rice (*Oryza sativa* L.) is widely grown in tropical and subtropical areas. It faces unexpected chilling damage, especially in some areas of temperate zones with moderate climate (Oliver et al., 2005; Nagasuga et al., 2011). The unpredictable cold snaps generally cause an average annual yield reduction of 5–10%, which could occasionally go up to 20–40%, despite many efforts that have been made to develop chilling-resistant cultivars (Oliver et al., 2005). Therefore, improving tolerance to extreme temperature through breeding or cultivation techniques is crucial to rice cultivation in the regions that experience extremely low temperatures.

During vegetative growth of rice, low temperature reduces the tillering rate, delays leaf development, and inhibits leaf elongation (Shimono et al., 2002). In some cases, these changes are accompanied by yellowing of leaves, slow growth, delayed crop

Abbreviations: F_v/F_m, Maximum quantum yield of PSII; Φ_{PSII}, Effective quantum efficiency of PSII; qP, Photochemical quenching; qN, Non-photochemical quenching; D, Antenna dissipative energy; P, Photochemistry dissipative energy; E_x, Excess energy; DCD, Dicyandiamide; SOD, Superoxide dismutase; CAT, Catalase; POD, Peroxidase; MDA, Malondialdehyde; AN, NH_4^+-N; NN, NO_3^--N; GN, Glycine-N; C, Control temperature; L, Low temperature.

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maturation, and poor development (Suzuki et al., 2008). Low temperature also directly or indirectly impacts an array of photosynthetic processes in chloroplasts, such as the thylakoid electron transport, carbon reduction cycle, and control of stomatal conductance (Lambers et al., 2008). This leads to the generation of reactive oxygen species (ROS) and further represses the net photosynthetic rate because of the accumulation of photo-energy (Alam and Jacob, 2002). The accumulation of ROS has detrimental effects on plant growth because they are highly destructive to lipids, nucleic acids, and proteins. To protect against ROS, higher plant species generally utilize a defense system involving anti-oxidative compounds or enzymes (Apel and Hirt, 2004). Previous studies have demonstrated that cold tolerant plants exhibit lower reductions in chlorophyll content (Kuk et al., 2002), higher leaf water content (Kuk et al., 2002), and higher anti-oxidative enzyme activity compared to the cold sensitive plants (Huang and Guo, 2005). Besides, many other metabolites and plant growth regulators, e.g. melatonin, proline, and spermidine, also play a crucial role in improving the resistance of plants to cold stress (Tan et al., 2012; Yamamoto et al., 2012).

Under low root-zone temperature, rice nitrogen (N) uptake ability is significantly reduced as a result of the reduced activity of enzymes and transporters (Feng et al., 2011). Plant N preferential uptake also varies with the soil or water temperature. Especially in some ecosystems with low temperature or lower N mineralization rate, amino acid uptake even constitutes a high proportion of plant total N economy compared to soil inorganic N (Näsholm et al., 2009). In addition, soil N forms have been demonstrated to be available for plant growth and photosynthesis, because of their different photo-energy consumption and reductant supply as well as the different chlorophyll content, stomatal conductance and chloroplast volume (Guo et al., 2002, 2007; Liu et al., 2013). A major difference in the soils under cold ecosystem, when compared to those under the tropical ecosystems, is the change in the form of available N; for example, the content of organic N increases whereas that of inorganic N decreases due to slow N mineralization rate. Plant photosynthesis is closely related to plant growth and dry matter accumulation. However, little is known, as of date, about the effects of amino acid N derived from organic fertilizers, on rice growth and leaf photosynthesis under cold stress.

In this study, we determined whether amino acid derived N can improve the cold tolerance of rice compared to the nitrate and ammonium derived N, and further decipher the possible reasons for such tolerance. To evaluate this physiological response in rice, a pot experiment was conducted under simulated cold stress condition in a green house, and the effects of different N forms on rice growth, N uptake, as well as on the physiological and photosynthetic traits during the vegetative period were examined under hydroponic cultivation with ammonium, nitrate and glycine as their sole N nutrition.

2. Materials and methods

2.1. Plant materials, cultivation, and cold-stress treatment

Rice (*Oryza sativa* L. cv. 'Zhongzheyou 1' indica hybrid rice) seedlings were grown hydroponically in an environmentally controlled growth chamber. After germination on moist filter paper, rice seeds were transferred to a 2.0-mmol L^{-1} $CaSO_4$ solution for germination. Three days later, rice seedlings were transferred to black plastic pots containing 1/4th -strength mixture of NO_3^- and NH_4^+ nutrient solution (for composition, see below). Ninety pots with internal radius of 6.0 cm and total depth of 12 cm were used in the experiment. Each pot was covered with a plastic cap, which had a 2.5-cm diameter hole drilled in the center for seedling to grow out of the pot. After three days in the pot, the seedlings were supplied with half-strength mixture of the nutrient solution. Subsequently, after three more days, the rice seedlings were supplied with full-strength nutrient solution for one week. The composition of the nutrient solution for hydroponic culture was as follows: macro-nutrients (mmol L^{-1}): 2.85 N as $(NH_4)_2SO_4$+$Ca(NO_3)_2$; 1.02 K as K_2SO_4 and KH_2PO_4; 0.32 P as KH_2PO_4, 1.65 Mg as $MgSO_4$; micro-nutrients (μmol L^{-1}): 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 (Li et al., 2012). Dicyandiamide (DCD, 0.2 mg L^{-1}) was added to each pot as the nitrification inhibitor. The solution pH was maintained at 5.50 ± 0.05 with the 1 mol L^{-1} HCl or 1 mol L^{-1} NaOH.

One week later, the seedlings were supplied with the nutrient solution containing either 2.85 mmol L^{-1} NH_4^+-N (AN), 2.85 mmol L^{-1} NO_3^--N (NN), or 2.85 mmol L^{-1} glycine-N (GN) as their sole N source, and others elements were provided as mentioned above. The treatments with each N source were done in 30 replicates. The seedlings from each N treatment were divided into two groups: seedlings in one group were grown in a PGW36 controlled environment growth chamber at 28 °C during daytime and 20 °C during night (the control temperature treatment, C), whereas seedlings in the other group were grown at 18 °C during daytime and 10 °C during night (the low temperature treatment, L). The six treatments groups were abbreviated as follows: NH_4^+-N at control temperature (AN-C), NH_4^+-N at low temperature (AN-L), NO_3^--N at control temperature (NN-C), NO_3^--N at low temperature (NN-L), glycine-N at control temperature (GN-C), and glycine-N at low temperature (GN-L). In ammonium-containing nutrient solution, Ca^{2+} was supplied as $CaCl_2$ (1.43 mmol L^{-1}). In addition, in the glycine treatments, the nutrient solutions contained 10 mg ampicillin L^{-1} to prevent the rapid de-amination of glycine, whereas in the ammonium and nitrate treatments, they contained 0.2 mg L^{-1} dicyandiamide (DCD). Each treatment had 15 replicates arranged in a completely randomized design (CRD) to avoid edge effect in the chamber. The nutrient solutions were changed every three days. The chamber was maintained with a 12-h photoperiod, 60% relative humidity, and a photosynthetic photon flux density (PPFD) of 1000 μmol m^{-2} s^{-1} during the photoperiod.

2.2. Gas-exchange and fluorescence measurement

Two weeks after the start of cold stress (35 days after sowing), light-saturated photosynthesis of the fourth newly expanded leaf in each treatment was measured from 09:00 to 15:00 h using a Li-Cor 6400 portable photosynthesis open system (Li-Cor Co. Ltd. UAS) in the temperature-controlled chamber. The leaf temperature during the measurements was maintained at 30 °C, with a PPFD of 1500 mmol m^{-2} s^{-1}. The ambient CO_2 concentration in the chamber was adjusted to 380 μmol mol^{-1} with a CO_2 mixture, and the relative humidity was maintained at 1.4–1.6 kPa. Data were recorded after equilibration to a steady state. The gas-exchange and fluorescence parameters were measured in five replicates for each treatment. These leaves were labeled, and the fluorescence measurements mentioned below were also made on the same leaves.

One day later, measurement of chlorophyll fluorescence was conducted on the above-mentioned labeled leaves with a portable pulse amplitude modulation fluorimeter (PAM-2000; Heinz Walz GmbH, Effeltrich, Germany). The chlorophyll fluorescence of the labeled leaf in the dark was monitored for approximately 30 min at 25 °C with an 800 ms pulse (8000 μmol m^{-2} s^{-1}, 20 KHz) of saturating light, and the maximum quantum yield of PSII (F_v/F_m, $F_v = F_m$-F_o) was determined after the fluorescence had reached a steady level. F_m and F_o represent the maximal and minimal fluorescence in the absence of non-photochemical quenching (dark-