Short communication

Exogenous Proline and Glycinebetaine Mitigate the Detrimental Effect of Salt Stress on Rice Plants

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Abstract

Exogenous application of proline and glycinebetaine (hereafter betaine) is often regarded as a shotgun approach for the protection and survival of plants under abiotic stresses including salinity. Here, we investigated effects of proline and betaine on hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and transpiration rate in salt-stressed rice plants. Generally, salt stress increased H_2O_2 and lipid peroxidation as indicated by MDA content and decreased transpiration rate in rice plants. The exogenous application of proline and betaine decrease H_2O_2 and MDA contents and increase transpiration rate in salt-stressed rice plants. It is suggested that exogenous proline and betaine mitigate the detrimental effects of salt stress by reducing H_2O_2 and lipid peroxidation levels and by increasing transpiration rate in rice plants.

Keywords*:* Proline; Betaine; Rice; Salt stress

Introduction

Soil salinity is a major environmental factors limiting plant growth and yield of most crops all over the world (Lauchli, 1986; Ashraf and Foolad, 2007). More than 20% of all irrigated land on earth is affected by salinization. Rice (*Oryza sativa* L.), as one of the world's most important cereal crops, provides the primary source of food and calories for about half of mankind (Khush, 2005). Although it is considered moderately sensitive, but most suitable plants for saline soil (Yeo and Flower, 1982).

Salt stress causes both ionic and osmotic effects in plants, leading to membrane disorganization, metabolic toxicity, and genesis of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), which may cause oxidative damage (Halliwell, 1987; Chaparzadeh et al., 2004). ROS have the potential to interact with all types of bio-molecules, such as DNA, proteins and lipids, leading to radical chain processes, peroxidation and membrane leakage (Halliwell and Gutteridge, 1989).

Proline and betaine are regarded as one of the most effective compatible solutes, plays an important role in plant salt tolerance by osmotic adjustment. The osmotic adjustment in the leaves and roots contribute to the maintenance of water uptake and cell turgor, allowing physiological processes such as photosynthesis and cell expansion (Serraj and Sinclair, 2002). In addition, exogenous proline and betaine mitigated salt stress via protecting membrane structures, up-regulating stress-protective proteins and reducing oxidation of lipid membranes in plants and cultured cells (Okuma et al., 2000; Khedr et al., 2003; Demiral and Turkan, 2004). In this study, we investigated the effects of exogenous proline and betaine on contents of H_2O_2, lipid peroxidation as well as transpiration rate in rice plants under saline conditions.

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Materials and Methods

Plant Material

Seeds of rice (*Oryza sativa* L. cv. Nipponbare) were collected from Field Science Center, Faculty of Agriculture, Okayama University, Japan. Seeds of rice were surface sterilized with 10% H_2O_2 and thoroughly rinsed with distilled water. The washed seeds were placed on water-soaked filter paper in a Petri dish. The Petri dishes were sealed with a strip of parafilm to prevent water vaporizations, and were incubated in a growth room(a 12-h-light/30°C and 12-h-dark/25°C regime and a photon influence rate of 80 μmol $m^{-2}s^{-1}$) for germination. After 7d of incubation, the seedlings were transferred from the dish to a floating net so that only the roots of the rice plants could be dipped in Kimura B solution, supplemented with 2.86 mg/Lof H_3BO_3, 1.80 mg/L of $MnCl_2$•$4H_2O$, 0.079 mg/L of $CuSO_4$•$5H_2O$, 0.126 mg/L of Na_2MoO_4 •$2H_2O$, and 0.220 mg/L of $ZnSO_4$•H_2O (Guo et al., 2005) , in a Wagner pot(r = 1/5,000). The plants were grown in growth room under the same conditions as describe for germination, the nutrient solution was changed every two days, and the pH was adjusted to 5.5 with 1N NaOH or 1N HCl. The rice plants were grown for 14 days followed by treatment with or without 25 mM NaCl in the presence and the absence of 1 mM proline or betaine for 12 h.

Determination of H_2O_2 Content

Hydrogen peroxide was measured by the 'DAB-staining method' as described previously with modification (Thordal-Christensen et al., 1997). Leaf samples were floated on assay solution for 2 h in the light (80 mmol m^{-2} s^{-1}); assay solution was prepared by dissolving 5 mM of KCl, 50 μM of $CaCl_2$, and 10 mM of MES-Tris (pH 6.15) in distilled water and leaf samples were then transferred to float on assay solution containing 1mg/ml of 3, 3-diaminobenzidine (DAB). After 3 h incubation leaves were boiled with ethanol (70%) for 10 min. Radish-brown color was observed and images were captured using an Olympus IX71S87 microscope equipped with a CS230 digital imaging color camera. DAB staining was evaluated using Adobe Photoshop Software. Data were expressed as relative units.

Lipid Peroxidation

Lipid peroxidation was determined by measuring malondialdehyde (MDA) formation using the thiobarbituric acid method as previously reported by Madhava Rao and Sreaty, 2000. Fifty mg of leaf sample was homogenized with 2 ml of 5% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000 x g for 15 min at 4°C and 1 ml of the supernatant was mixed with 2 ml of 20% TCA containing 0.5% thiobarbituric acid (TBA). The mixture was heated at 95°C for 25 min and then cooled quickly on an ice bath. The mixture was centrifuged for 5 min at 7500 x g and the absorbance of the supernatant was measured at 532 nm. Measurements were corrected for unspecific value by subtracting the absorbance of that supernatant at 600 nm using a spectrophotometer (model UV-2400 PC, Shimadzu, Kyoto). The amount of MDA was calculated by using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Transpiration Rate

Transpiration rates by second leaves of the rice plants were measured using steady state porometer (model: LI-1600, LI-COR, inc. Lincoln, Nebraska, USA).

Statistical Analysis

Significant of differences between mean values were assessed using analysis of variance (ANOVA) with the Student-Neuman-Keuls multiple-range test. Differences at $p < 0.05$ were considered significant.

Results and Discussion

Hydrogen peroxide is a toxic compound which is injurious to the cell and excessive accumulation of H_2O_2 is one of the indicators of oxidative stress (Hasanuzzaman et al., 2011). We investigated whether proline and betaine could inhibit NaCl-induced H_2O_2 accumulation. The leaves were incubated with DAB and the stains developed by DAB in the presence of H_2O_2 were quantified (Figure 1A). Salt stress resulted in a significant increase (38%) in H_2O_2 content. Proline and betaine supplemented salt-stressed rice plants also showed continual increase in H_2O_2 content when compared with control. However, the level of H_2O_2 content in salt stress plants was significantly lower than those of plants treated with salt

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alone. Accumulation of H_2O_2 in the context of salinity damage has been documented for many plant species (Mandhania et al., 2006; Weisany et al., 2012; Hasanuzzaman et al., 2014; Rohman et al., 2015). A similar result was reported by Hasanuzzaman et al. (2014) and Rohman et al. (2015), where exogenous proline and betaine reduce H_2O_2 level in rice and maize plant induced by salt stress, respectively, indicating that proline and betaine serve as scavengers of ROS under salt stress conditions (Ashraf and Foolad, 2007).

Lipid peroxidation is considered the most damaging process caused by ROS attack on polyunsaturated fatty acids in the membrane, leading to loss of membrane integrity and MDA amount is often used to monitor the level of lipid peroxidation under oxidative stresses (El-Baky et al., 2003; Garg and Manchanda, 2009). The lipid peroxidation in the leaves of the rice plants was determined as the content of MDA is shown in Figure 1B. Salt stress caused a significant increase (34%) in MDA content compare to control which was in agreement with previous reports (Lin et al., 2006; Azooz et al., 2009; Hasanuzzama et al., 2014; Rohman et al., 2015). Application of proline and betaine reduced MDA content in salt-stressed plants by 29% and 31%, respectively when compared with those of salt stressed plants not applied with proline and betaine (Figure 1B). It was reported that exogenous proline and betaine mitigate the oxidative damage by enhanced the stability of lipids in membranes (Demerial and Turkan, 2004; Haque et al., 2007; Banu et al., 2009; Hasanuzzaman et al., 2014; Rohman et al., 2015).

Stress, in general, is known to alter plant-water relations (Barcelo and Poschenrieder, 1990) which may affect water uptake, ascent of sap (Poschenrieder and Barcelo, 2004) and ultimately results in decreased plant growth. Transpiration rate in leaves of rice plants at 3, 6, 9 and 12 h was measured using steady state porometer (Figure 2). When salt stress was induced, transpiration was reduced as a reaction to the osmotic shock. This may be due to the fact that lowered water potentials in the root can trigger a signal from root to shoot. Application of proline and betaine increased transpiration rate within 3 h of treatment under salt-stressed plants (Figure 2). The decrease in transpiration (Zheng et al., 2001; Sharma et al., 2005; Moradi and Ismail, 2007) and leaf relative water content (Hu and Hu, 2012; Hasanuzzaman et al., 2014) in salt-stressed plants suggested that salt stress decreased the water flow from roots to leaves and caused an adverse effect on the growth of rice plants. Exogenous proline or betaine increased transpiration rate (Figure 2) and relative water content (Hu and Hu., 2012; Hasanuzzaman et al., 2014) in salt-stressed plants. This suggested that proline and betaine could increase the hydraulic conductivity, which may enhance the water flow from roots to leaves and eventually increase the relative water content and transpiration rate under salt stress (Prisco, 1980; Shaddad, 1990; Ali et al., 2007; Hu and Hu, 2012).

There are many reports on improving water relations, photosynthetic abilities and growth, and a protective role in salt induced oxidative stress by exogenous proline and betaine in salt-stressed rice plants (Demiral and Turkan, 2004; Chaum et al., 2006; Hasanuzzaman et al., 2014). In the present study, we found that exogenous application of proline and betaine was positively related to protect rice plants against salt stress-induced oxidative damage and transpiration rate, leading to mitigate detrimental effects of salt stress. Petcu et al. (2007) also suggested the use of transpiration as a screening tool for saline tolerance of alfalfa genotypes. Therefore, we hypothesis that transpiration is an important salt resistance attribute in rice. It has been shown that total dry matter production is linearly and positively related to crop transpiration (Fraga et al., 2010). This relationship is derived from the fact that the control of both transpiration and CO_2 exchange is dependent on stomatal activity. However, possible effects of exogenous proline and betaine on stomatal movements in rice under salt stress conditions have not yet been studied and need to be elucidated.

Conclusions

Exogenous application of proline and betaine was effective in mitigating the detrimental effects of salt stress on rice plants by reducing H_2O_2 and oxidation of membrane lipid and by increasing transpiration rate under salt stress.

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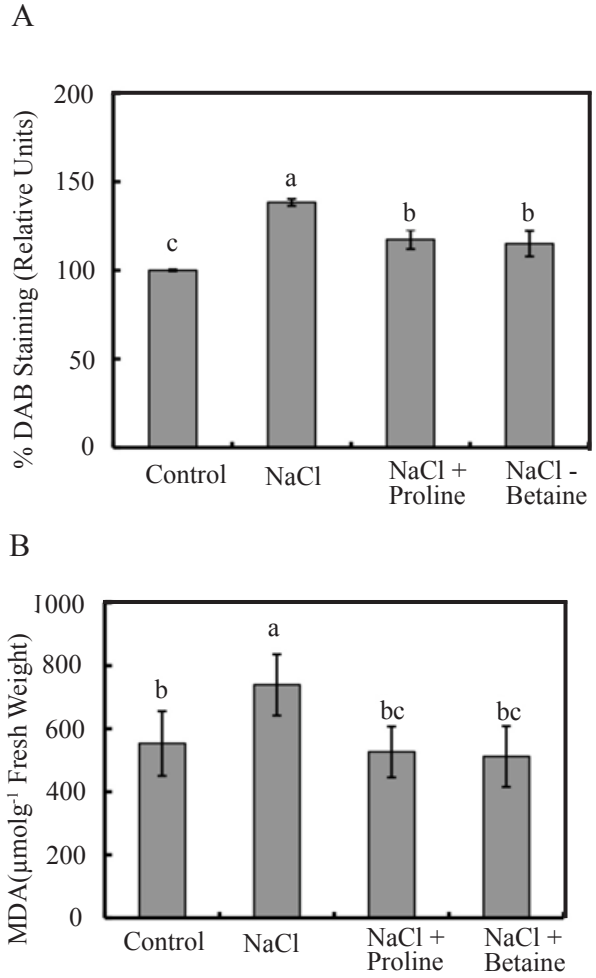

Figure 1 H_2O_2 and lipid peroxidation (represented by MDA) in the rice plants under the saline condition. A, Increasing DAB staining in the rice leaves indicates the higher H_2O_2 concentrations and B, MDA contents in the rice plants treated with 25 mM NaCl in the presence and the absence of 1 mM proline or betaine for 12h. Each Value was obtained from more than three experiments. Error bars represent standard deviation. Bars with different letters are significantly different at $p < 0.05$.

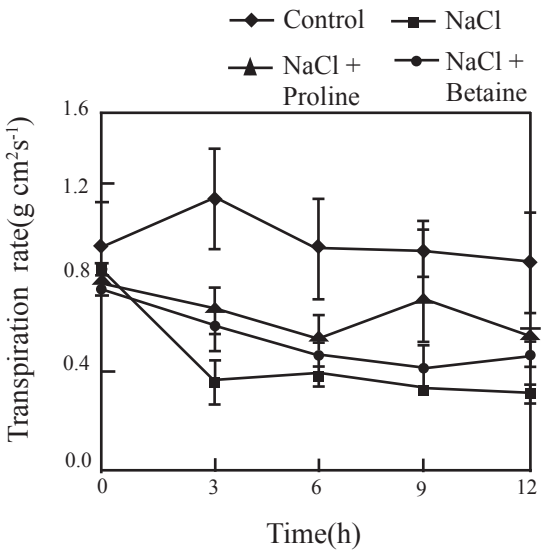

Figure 2 Transpiration rate in the rice plants under the saline condition. Transpiration rate in the rice plants treated with 25 mM NaCl in the presence and the absence of 1 mM proline or betaine for 12h. Each Value was obtained from more than three experiments.

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