Journal of Plant Physiology 169 (2012) 596–604

Contents lists available at SciVerse ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

Exogenous proline and trehalose promote recovery of rice seedlings from salt-stress and differentially modulate antioxidant enzymes and expression of related genes

Noppawan Nounjan[a], Phan Tuan Nghia[b], Piyada Theerakulpisut[a,*]

[a] Genomics and Proteomics Research Group for Improvement of Salt-tolerant Rice, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
[b] Key Laboratory of Enzyme and Protein Technology, Faculty of Biology, Hanoi University of Science, Hanoi, Viet Nam

ARTICLE INFO

Article history:
Received 17 August 2011
Received in revised form 11 January 2012
Accepted 11 January 2012

Keywords:
Antioxidant enzymes
Proline
Rice
Salt stress
Trehalose

ABSTRACT

Proline (Pro) and Trehalose (Tre) function as compatible solutes and are upregulated in plants under abiotic stress. They play an osmoprotective role in physiological responses, enabling the plants to better tolerate the adverse effects of abiotic stress. We investigated the effect of exogenous Pro and Tre (10 mM) in seedlings of Thai aromatic rice (cv. KDML105; salt-sensitive) during salt stress and subsequent recovery. Salt stress (S, NaCl) resulted in growth reduction, increase in the Na^+/K^+ ratio, increase in Pro level and up-regulation of Pro synthesis genes (pyrroline-5-carboxylatesynthetase, *P5CS*; pyrroline-5-carboxylate reductase, *P5CR*) as well as accumulation of hydrogen peroxide (H_2O_2), increased activity of antioxidative enzymes (superoxide dismutase, SOD; peroxidase, POX; ascorbate peroxidase, APX; catalase, CAT) and transcript up-regulation of genes encoding antioxidant enzymes (*Cu/ZnSOD*, *MnSOD*, *CytAPX*, *CatC*). Under salt stress, exogenous Pro (PS; Pro + NaCl) reduced the Na^+/K^+ ratio, further increased endogenous Pro and transcript levels of *P5CS* and *P5CR*, but decreased the activity of the four antioxidant enzymes. The transcription of genes encoding several antioxidant enzymes was upregulated. Exogenous Tre (TS; Tre + NaCl) also reduced the Na^+/K^+ ratio and strongly decreased endogenous Pro. Transcription of *P5CS* and *P5CR* was upregulated, the activities of SOD and POX decreased, the activity of APX increased and the transcription of all antioxidant enzyme genes upregulated. Although exogenous osmoprotectants did not alleviate growth inhibition during salt stress, they exhibited a pronounced beneficial effect during recovery period showing higher percentage of growth recovery in PS (162.38%) and TS (98.43%) compared with S (3.68%). During recovery, plants treated with PS showed a much greater reduction in endogenous Pro than NaCl-treated (S) or Tre-treated plants (TS). Increase in CAT activity was most related to significant reduction in H_2O_2, particularly in the case of PS-treated plants. Advantageous effects of Pro were also associated with increase in APX activity during recovery.

Introduction

Soil salinity is one of the most important abiotic stress problems which inhibit growth and reduces productivity of crops including rice, tomato, chili and potato especially in drier parts of many countries around the globe. In Thailand, 62% (6.08×10^6 tons/ha) of rice-growing areas are located in the Northeastern part of the country, but due to water shortage and soil salinity problems arising from the presence of underground salt domes, rice productivity from this area has been relatively low. In the year 2010, rice productivity from the Northeast was 2×10^3 tons/ha compared to 3.3×10^3 tons/ha from the central plain which hardly experiences water shortage and has no saline soils (Thai Rice Exporters Association, 2011; http://www.thairiceexporters.or.th/production.htm).

Salt stress arises from the combination of osmotic and ion toxicity effect (primary effect), and oxidative stress (secondary effect).

Abbreviations: APX, ascorbate peroxidase; C, nutrient solution without Pro/Tre and NaCl; CAT, catalase; GB, glycinebetaine; GSA, glutamate semialdehyde; H_2O_2, hydrogen peroxide; KDML105, *Oryza sativa* cv. Khao Dawk Mali 105; P, 10 mM Pro; P5C, pyrroline-5-carboxylate; P5CDH, pyrroline-5-carboxylate dehydrogenase; P5CR, pyrroline-5-carboxylate reductase; P5CS, pyrroline-5-carboxylatesynthetase; PDH, proline dehydrogenase; POX, peroxidase; Pro, proline; PS, 10 mM Pro plus 100 mM NaCl; S, 100 mM NaCl; sqRT-PCR, semi-quantitative reverse transcriptase-polymerase chain reaction; SOD, superoxide dismutase; T, 10 mM Tre; T6P, Tre-6-phosphate; TPP, Tre-6-phosphate phosphatase; TPS, Tre-6-phosphate synthase; Tre, trehalose; TS, 10 mM Tre plus 100 mM NaCl.

* Corresponding author. Tel.: +66 43 342908; fax: +66 43 364169; mobile: +66 89 6231777.
E-mail addresses: 5150200091@stdmail.kku.ac.th (N. Nounjan), phantn@fpt.vn (P.T. Nghia), piythe@kku.ac.th (P. Theerakulpisut).

0176-1617/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jplph.2012.01.004

Salts in the soil water inhibit plant growth primarily by reducing the ability of the plant to take up water thus leading to slower growth. Secondarily, a buildup of toxic level of Na^+ and Cl^- and inhibition of K^+ uptake severely inhibits several enzymes requiring K^+ as cofactors leading to a whole range of metabolic impairment (Munns et al., 2006). Under salinity stress, an increase in the biosynthesis of compatible solutes such as Pro (Pro), ectoine, glycine betaine, sorbitol and Tre (Tre) protects cells against hyperosmotic stress. The high concentration of compatible solutes is able to balance the concentration of salts outside the cell on one side, and on the other, to counteract the high concentrations of Na^+ and Cl^- in the vacuole (Türkan and Demiral, 2009). Under salinity and other abiotic stresses plants can generate reactive oxygen species (ROS) such as superoxide anion ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2). These ROS are strongly reactive because they can interact with essential macromolecules and metabolites causing cellular damage. In order to protect cells and tissue from oxidative damage plants must produce non-enzymatic antioxidants such as glutathione and ascorbate as well as antioxidant enzymes including peroxidase (POX; EC1.11.1.7), superoxide dismutase (SOD; EC1.15.1.1), ascorbate peroxidase (APX; EC1.11.1.1) and catalase (CAT; EC1.11.1.6) to defend against oxidative stress (Ashraf, 2009).

Pro is the most common osmolyte accumulating in plants in response to various stress conditions. It offers a wide range of protective roles including osmotic adjustment, stabilizer for cellular structure and reduction of damage to the photosynthetic apparatus. The level of Pro accumulation in plants varies from species to species. The importance of Pro in enhancing plant stress tolerance has recently been substantiated through a transgenic approach. Transgenic rice expressing the *P5CS* gene from mothbean showed an enhanced accumulation of *P5CS* mRNA level, Pro content and higher tolerance to drought and salt stress (Su and Wu, 2004).

Tre is a non-reducing disaccharide found in many organisms. It is an essential component of the mechanisms that coordinate metabolism with plant growth adaptation and development (Paul, 2007). Tre accumulation influences the alteration of sugar metabolism leading to an osmoprotectant effect under stress (Djilianov et al., 2005). In transgenic rice which received the *ots*A and *ots*B genes (*TPS* and *TPP* in higher plant) from *Escherichia coli*, Tre accumulated 3–10 fold higher when compared to the wild type and overproduction of Tre increased tolerance to abiotic stresses (Garg et al., 2002). Ge et al. (2008) demonstrated that *OsTPP1* overexpression in rice enhanced tolerance to salt and cold stress.

Exogenous osmoprotectants have been reported to have osmoprotective roles in abiotic stress response and have been suggested as an alternative approach to improve crop productivity under saline conditions (Nakayama et al., 2005). Exogenous application of Pro has been reported to offer beneficial effects to plants under stress conditions (Ashraf and Foolad, 2007). For example, in tobacco under salt stress, adding exogenous Pro to cell suspension culture alleviated the effect of salt stress and increased the activities of antioxidant enzymes (Hoque et al., 2007). Moreover, exogenous Pro decreased protein carbonylation and enhanced antioxidant defense and methylglyoxal detoxification systems (Hoque et al., 2008). Pretreatment of maize with 10 mM Tre relieved the damaging effects of salinity stress on the metabolic pathways such as Hill-reaction activity, photosynthetic pigments and nucleic acids content (Zeid, 2009). Tre pretreatment of winter wheat protected thylakoid membranes from heat damage, maintained cell membrane integrity and reduced ROS accumulation from heat stress (Luo et al., 2010).

Thai aromatic rice (cv. KDML105) is a well-known economically important Thai cultivar highly recognized in the international market (known as Thai Hom Mali Rice) as the world's best quality aromatic rice. However, KDML105 is sensitive to salt stress, especially during the seedling stage, giving low yield and poor grain milling quality when it is grown under saline soils (Gregorio et al., 1997; Summart et al., 2010). The aim of this work was to test the effects of exogenous Pro and Tre on physiological responses in seedlings of KDML105 during salt stress and recovery period. Few reports have addressed the effects of these osmoprotectants on modification of physiological responses during salt stress in rice. This work provides additional information on the roles of exogenously applied osmoprotectants in modifying responses of rice during salinity stress as well as during the recovery period.

Materials and methods

Plant materials and treatments

Seeds of rice (*Oryza sativa* L. cv. KDML105) were germinated in distilled water for 5 d at room temperature (RT), and then transferred to plastic chambers containing Yoshida solution (Yoshida et al., 1976) under natural sunlight in a greenhouse for 28 d during which the solutions were renewed every 4 d. The plants were then divided into six treatment groups by addition of the following solutions into Yoshida solution for 6 d as follows: Yoshida solution without Pro/Tre and NaCl (C), 100 mM NaCl (S), 10 mM Pro (P), 10 mM Pro plus 100 mM NaCl (PS), 10 mM Tre (T) and 10 mM Tre plus 100 mM NaCl (TS). The use of 10 mM Pro and Tre was based on the previous report of Garcia et al. (1997) and a preliminary experiment in our laboratory (unpublished data, 2006). The experiment was set up according to a completely randomized design with 5 replications. After 6 d treatment the plants were then allowed to recover for 5 d by replacing the treatment solutions with Yoshida solution. Rice plants were harvested twice; the first, after the 6 d stressed period and the second, after the 5 d recovery period. Plants were analyzed for fresh and dry weights, Na^+/K^+ ion concentration, Pro accumulation, H_2O_2 content, total protein, activity of antioxidant enzymes (POX, SOD, APX and CAT) and gene expression (genes encoding Pro synthesis and antioxidant enzymes).

Growth parameters and ion concentration

Plant fresh weight was determined and then the plants were dried in a hot-air oven at 70 °C for 4–5 d until the dry weight was stabilized. The dried plant materials were ground to fine powder. Dried samples (0.1 g) were subjected to chemical analyses by digesting in 10 mL of nitric acid at 300 °C, 5 mL perchloric acid at 200 °C and 20 mL of 6 M hydrochloric acid. The concentration of Na and K ions were analyzed using an Atomic Absorption Spectrometer (Model GBC 932 AAA, England).

Determination of Pro and H_2O_2 content

The method described by Bates et al. (1973) was applied to quantify Pro content. Briefly, leaf samples (0.1 g) were homogenized in 5 mL of 3% sulfosalicylic acid then filtered. Two mL of filtrate was mixed with 2 mL of ninhydrin reagent (1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL 6 M phosphoric acid) and 2 mL of glacial acetic acid. The reaction mixture was heated at 100 °C for 1 h and then placed on ice for 20 min before being extracted with 4 mL of toluene. The absorbance of the red chromophore in the toluene fraction was measured at 520 nm and the amount of proline was determined by comparison with a standard curve. For measurement of H_2O_2, leaf tissues (0.1 g) were homogenized with 1 mL of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 12,000 × *g* for 15 min. The supernatant (0.5 mL) was added to 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide. The absorbance of H_2O_2 was determined using a