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Alleviation of the adverse effects of salinity stress using trehalose in two rice varieties

M.M.-S. Abdallah [a,*], Z.A. Abdelgawad [b], H.M.S. El-Bassiouny [a]

[a] Botany Department, Agriculture and Biology Division, National Research Centre, Dokki, Giza, 33 El Bouhouth st, P.O. 12622, Egypt
[b] Botany Department, Women's College, Ain Shams University, Cairo, Egypt

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ABSTRACT

A possible survival strategy of plants under effects of salinity is to use some compounds that could help plants to alleviate salt stress effect. The effect of exogenously application of trehalose as seed soaking prior to irrigation with different salinity levels (0, 30 and 60 mM NaCl) to rice (*Oriza sativa* L.) varieties (Giza 177 and Giza 178) was investigated. Salinity stress decreased photosynthetic pigments and total carbohydrate, concomitantly with increasing total soluble sugars content, trehalose and proline content for both rice varieties. The activities of SOD, CAT and POX were increased with increasing salinity level. Soaking rice seeds with 25 mM of trehalose (Tre) could alleviate the harmful effects of salinity stress. Moreover, a higher solute concentration contributing to osmotic adjustment and the higher antioxidant enzymes activity were observed in shoots of salinity treated Giza 178 than those of Giza 177.

1. Introduction

Increasing food production in the conditions of an increasing world population is of a major concern. Abiotic stresses can directly or indirectly affect the physiological status of an organism by altering its metabolism, growth, and development.

Salinity is one of the major abiotic stresses in arid and semiarid regions that substantially limiting crop production and reduces the average yield of major crops by more than 50% (Bray et al., 2000). High concentrations of salt resulting from natural processes or disarrangement in irrigated agriculture result in inhibition of plant growth and yield (Tester and Bacic, 2005). Salinity primarily imposes on plants an osmotic stress and secondarily ion toxicity stress. Excess salt in the soil may adversely affect plant growth either through osmotic inhibition of water uptake by roots or specific ion effects. Specific ion effects may cause direct toxicity or alternatively, the insolubility or competitive absorption of ions may affect plant nutritional balances (Silva et al., 2008). Moreover, the capacity of plants to activate antioxidant defense is also showed to be closely associated with plant tolerance to salinity in barley and legume (Chen and Polle, 2010). Salt stress has toxic effects on plants and lead to metabolic changes, like loss of chloroplast activity, decreased photosynthetic rate and increased photorespiration rate which leads to an increased reactive oxygen species (ROS) production (Parida and Das, 2005). Therefore, the development of methods and strategies to ameliorate the deleterious effects of salt stress on plants has received considerable attention. Enhancing stress tolerance in plants has major implications in agriculture and horticulture (Senaratna et al., 2000). Salt tolerance is a complex trait that is controlled by multiple genes and involves various biochemical and physiological mechanisms (Zhang and Shi, 2013). Osmotic adjustment is an effective mechanism for enduring salt stress-induced hyperosmotic stress (Chen and Jiang, 2010).

A common response of organisms to drought, salinity, and low-temperature stresses is the accumulation of sugars and other compatible solutes (El-Bassiouny and Bekheta, 2005; Khalil et al., 2009). These compounds serve osmoprotectants and in some cases, stabilize biomolecules under stress conditions (Theerakulpisut and Phongngarm, 2013). One such compound is trehalose (Tre), a non-reducing disaccharide of glucose, consisting of two units of glucose (α-D-glucopyranosyl-1,1-α-D-glucopyranoside) is widely spread in a variety of organisms: bacteria, yeast, fungi, lower and higher plants, as well as insects and other invertebrates (Elbein et al., 2003). Trehalose, plays an important physiological role as an abiotic stress protectant in a large number of organisms, including bacteria, yeast and plant (Almeida et al., 2007). Luo et al. (2010) suggested that Tre is likely to function through its ability to scavenge reactive oxygen species, conferring protection to the machinery of protein synthesis. Tre has the added advantage of being a signaling and antioxidant molecule. Tre also acts as an elicitor of genes involved in detoxification and stress response (Bae et al., 2005).

* Corresponding author.
E-mail address: maha_eg1908@yahoo.fr (M.M.-S. Abdallah).

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Trehalose itself is eventually hydrolyzed by trehalase into two molecules of glucose (Iordachescu and Imai, 2008). Trehalose has been shown to stabilize dehydrated enzymes, proteins, and lipid membranes efficiently, as well as protect biological structures from damage during desiccation. The importance of trehalose in stress conditions compared with other sugars can be explained by several unique physical properties, which include high hydrophilicity, chemical stability, and the absence of internal hydrogen bond formation that account for the principal ability of trehalose for protein stabilization. It has been proposed that in the absence of water, trehalose preserves membrane or protein structures by forming an amorphous glass structure and interacting through hydrogen bonds with polar phospholipids head groups or with amino acids (Crowe et al., 1984). Thereby trehalose is helping the protein to keep in shape and concentrate the remaining water next to the protein (Schiraldi et al., 2002) . Recently, there is a focus of interest in the role of Tre as it improves the performance of plants under drought, nutrition element or salinity (Iordachescu and Imai, 2008; Paul et al., 2008). The underlying mechanism, by which Tre improves plant response to salinity, and other adverse environmental factors, is still unclear (Paul et al., 2008).

Rice, one of the world's most important staple crops, can be cultivated in most climatic conditions but is relatively intolerant to salinity. Salinity affects all aspects of rice growth to varying degrees at all stages of growth from germination to maturity. Increased salt tolerance of elite rice cultivars is needed to sustain food production in many drier regions of the world especially where the area of salt-affected soils are ever expanding due to increasing temperature and land clearing.

Thus, the main objective of the present study was to examine whether the adverse effects of salt stress on two rice varieties could be mitigated by exogenous application of trehalose as a seed soaking and how far it regulates the plant antioxidant enzyme system and some metabolites.

2. Materials and methods

Two varieties of rice (*Oriza sativa* L.) Giza178 and Giza177 were obtained from the Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt. Seeds were previously sterilized and after washing several times with distilled water, the seeds of each variety were divided into two groups. The first group was left soaked in distilled water (control), and the second group was pre-soaked in 25 mM trehalose for 12 h. Then the seeds of the two varieties were grown in pots (35 cm diameter and 40 cm depth); each pot contained 7 kg soil. Soil characteristics were: sandy loam in texture, sand 84.2%, silt 12.9%, clay 2.9%, pH 7.7, EC 0.5dSm^{-1} and organic matter 1.2%. Seven seeds per pot and were thinned to four after two week post planting. Five replicates were used for each treatment.

Seedlings of the 1st and the 2nd groups for both varieties of rice plants were irrigated with different levels of saline solutions (0, 30, 60 mM NaCl). Each of the previous groups of both varieties was divided into three subgroups according to irrigation with different levels of saline solutions (0, 30, 60 mM NaCl). Every treatment consisted of 5 replicates distributed in a completely randomized design system. Pots were irrigated with equal amount volumes of the two various salinity levels. Irrigation was run as follows 3 times with saline solutions and one with tap water. The plant samples were taken at 35 days after sowing for the estimation of growth parameters (shoot length, shoot fresh and dry weights and relative water contents (RWC), biochemical analysis of photosynthetic pigments, total soluble sugars, total carbohydrates, trehalose, proline and oxidative enzyme activities (peroxidases (POX), catalase (CAT), and superoxide dismutase (SOD).

2.1. Chemical analysis

2.1.1. Photosynthetic pigments

Total chlorophyll a and b and carotenoid contents in fresh leaves were estimated using the method of Lichtenthaler and Buschmann (2001). The fresh tissue was extracted in 80% acetone. The optical density (OD) of the solution was recorded at 662, 645 and 470 nm for chlorophyll a, b and carotenoids using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan).

2.1.2. Total carbohydrate

Determination of total carbohydrates was carried out according to Herbert et al. (1971). A known mass (0.2–0.5 g) of dried tissue was added in 10 ml of sulphuric acid (1 N). The tube was sealed and placed overnight in an oven at 100 °C. The total sugars were determined colorimeterically according to the method of Smith et al. (1956) as follows: An aliquot of 1 ml of sugar solution was mixed with 1 ml of 5% aqueous phenol solution followed by 5.0 ml of concentrated sulphuric acid. The tubes were thoroughly shaken then placed in a water bath at 23–30 °C for 20 min. The optical density of the developed color was measured at 490 nm using Shimadzu spectrophotometer.

2.1.3. Total soluble sugars (TSS)

Total soluble sugars (TSS) were extracted by overnight submersion of dry tissue in 80% (v/v) ethanol at 25 °C with periodic shaking, and centrifuged at 600 g. The supernatant was evaporated till completely dried then dissolved in a known volume of distilled water to be ready for determination of soluble carbohydrates (Homme et al., 1992). TSS were analyzed by reacting 0.1 ml of ethanolic extract with 3.0 ml freshly prepared anthrone in boiling water bath for ten minutes and reading the cooled samples at 625 nm using a Shimadzu Spectrocolorimeter (Yemm and Willis, 1954).

2.1.4. Trehalose

Trehalose content was extracted according to the method described by (Lynch et al., 2010). Rice tissues were mixed with 5 mL in 80% methanol and incubated at 85 °C in a water bath for1 h. Samples were then centrifuged at 980 g for 2 min and their supernatants collected. This supernatant was evaporated in a vacuum oven set at 100 °C over night and the dry residue dissolved in 2 mL of deionized water. For trehalose quantitation, the anthrone reaction was used based on (Umbreit et al., 1972). Briefly, 0.5 mL each of the trehalose solutions was mixed with 5 mL of 66% sulphuric acid containing 0.05% w/v anthrone and incubated at 100 °C for 15 min. After cooling, sample absorbance was measured at 620 nm and compared to a trehalose concentration versus absorbance standard curve to evaluate trehalose concentration.

2.1.5. Proline determination

Proline was assayed according to the method described by Bates et al. (1973). 2 ml of proline extract, 2 ml of acid ninhydrin and 2 ml of glacial acetic acid were added and incubated for 1 h in a boiling water bath followed by an ice bath. The absorbance was measured at 520 nm. A standard curve was obtained using a known concentration of authentic proline.

2.1.6. Assay of enzyme activities

Enzyme extracts were collected following the method described by Chen and Wang (2006). Leaf tissues were homogenized in an ice-cold phosphate buffer (50 mM, pH 7.8), followed by centrifugation at 8000 rpm and 4 °C for 15 min. The supernatant was used immediately to determine the activities of enzymes.

2.1.6.1. Peroxidase. (POX, EC 1.11.1.7) activity was spectrophotometrically assayed by the method of Kumar and Khan (1982). The reaction mixture used for estimating the peroxidase enzyme (POX) contained 2 ml of 0.1 M phosphate buffer (pH 6.8), 1 ml of 0.01 M pyrogallol, 1 ml of 0.005 M H_2O_2 and 0.5 ml of the enzyme extract. The solution was incubated for 5 min at 25 °C after which the reaction was terminated by adding 1 ml of 2.5 N H_2SO_4. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a