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Antioxidant Defense Mechanisms of Salinity Tolerance in Rice Genotypes

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Abstract: In order to elucidate the role of antioxidant responses in salinity tolerance in rice genotypes under salt stress, experiments were conducted using four rice varieties, including salt-sensitive BRRI dhan 28 and three salt-tolerant varieties BRRI dhan 47, BINA dhan 8 and BINA dhan 10. Thirty-day-old rice seedlings were transplanted into pots. At the active tillering stage (35 d after transplanting), plants were exposed to different salinity levels (0, 20, 40 and 60 mmol/L NaCl). Salt stress caused a significant reduction in growth for all the rice genotypes. Growth reduction was higher in the salt-sensitive genotype than in the salt-tolerant ones, and BINA dhan 10 showed higher salt tolerance in all measured physiological parameters. The reduction in shoot and root biomass was found to be minimal in BINA dhan 10. Chlorophyll content significantly decreased under salt stress except for BINA dhan 10. Proline content significantly increased in salt-tolerant rice genotypes with increased salt concentration, and the highest proline content was obtained from BINA dhan 10 under salt stress. Catalase and ascorbate peroxidase activities significantly decreased in salt-sensitive genotype whereas significantly increased in salt-tolerant ones with increasing salt concentration. However, salt stress significantly decreased guaiacol peroxidase activity in all the rice genotypes irrespective of salt tolerance. K^+/Na^+ ratio also significantly decreased in shoots and roots of all the rice genotypes. The salt-tolerant genotype BINA dhan 10 maintained higher levels of chlorophyll and proline contents as well as catalase and ascorbate peroxidase activities under salt stress, thus, this might be the underlying mechanism for salt tolerance.

Key words: antioxidant enzyme; salinity; chlorophyll content; proline content; K^+/Na^+ ratio; rice

Climate change is a serious environmental threat that causes sea level rise and thereby affects coastal areas of Bangladesh. Salinity is one of the major abiotic factors, limiting crop production worldwide. Approximately 7% of the world's total land area, 20% of the world's cultivated land area and nearly 50% of the world's irrigated land area are affected by salinity (Zhu, 2001). In Bangladesh, about 1.06 million hectares of arable lands are affected by soil salinity (SRDI, 2010). Salinity is a serious threat to rice production in the southern part of Bangladesh. Salinity imposes both ionic toxicity and osmotic stress to plants (Hasegawa et al, 2000; Zhu, 2003). Salt stress disturbs cytoplasmic K^+/Na^+ homeostasis, causing a decrease in K^+/Na^+ ratio in the cytosol (Zhu, 2003). Accumulation of excess Na^+ and Cl^- causes ionic imbalance that may impair the selectivity of root membranes and induce K^+ deficiency in plants.

Rice is the most important cereal crop in the world, yielding one-third of the total carbohydrate sources. The total rice growing area in Bangladesh is about 10.83 million hectares, leading to the production of 33.54 million metric tons of rice. On the contrary, rice yield is very low in the salt soil. To increase rice yield per acre, it is imperative to know about physiological and biochemical changes in plants under salt stress.

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Plants have evolved a variety of adaptive mechanisms to respond to salt stress. One of the main adaptive mechanisms is the accumulation of compatible solutes (Sharma and Dietz, 2006; Ashraf and Foolad, 2007), and proline is the most common one. Increased levels of endogenous proline accumulation in plants is correlated with enhanced salt tolerance (Hasegawa et al, 2000; Ashraf and Foolad, 2007; Boscaiu et al, 2012; Sripinyowanich et al, 2013), and induce oxidative stress tolerance by modulating the activities of antioxidant enzymes (Saeedipour, 2013). Proline accumulation also plays a regulatory role during plant growth under salt stress (Mattioli et al, 2008).

Salinity induces the production of reactive oxygen species (ROS) in plant cells (Hasegawa et al, 2000; Banu et al, 2009, 2010). ROS can act as signaling molecules, mediating many key physiological processes. Excess production of ROS is toxic to plants and causes oxidative damage to cellular constituents, leading to cell death (Noctor and Foyer, 1998; Banu et al, 2009, 2010). Plants possess enzymatic and non-enzymatic antioxidant defense systems to protect cells against the damaging effects of ROS. The major ROS-scavenging antioxidant enzymes are catalase (CAT), guaiacol peroxidase (POX) and ascorbate peroxidase (APX). Salt stress shows different effects on the components of antioxidant defense systems in plants (Hasegawa et al, 2000; Mittova et al, 2003; Demiral and Turkan, 2005; El-Shabrawi et al, 2010; Hasanuzzaman et al, 2014).

There is increasing evidence that salt stress has a significant effect on physiological and biochemical attributes of plants. Better understanding of physiological and biochemical characteristics of plants is vital for improving salt tolerance under salinity. To clarify the physiological and biochemical responses of salt-sensitive and salt-tolerant rice genotypes, we investigated the plant growth, chlorophyll content, proline content and activity of antioxidant enzymes in four contrasting rice genotypes exposed to salt stress, which can be used as index for *in vitro* salt tolerance in rice.

MATERIALS AND METHODS

Rice materials and experimental design

Four rice genotypes, including one salt-sensitive variety (BRRI dhan 28) and three salt-tolerant varieties (BRRI dhan 47, BINA dhan 8 and BINA dhan 10), were exposed to different NaCl levels (0, 20, 40 and 60 mmol/L). The experiment was laid out in a randomized complete block design with four replications. Considerable spacing was maintained among the pots for the ease of management practices.

Pot preparation and management practices

Pot experiments were carried out at a net-house (average temperature 24 ºC and relative humidity 60%) of the Department of Soil Science, Bangladesh Agricultural University, Bangladesh to investigate the effects of salt stress on the growth as well as the physiological and biochemical characteristics of salt-sensitive and salt-tolerant rice.

Soils were collected from the Soil Science Field Laboratory, Bangladesh Agricultural University. The initial soil sample was silt loam having exchangeable Na 0.383 meq/100 g and exchangeable K 0.082 meq/100 g. Each 15 L plastic pot was filled with 8 kg soil and 5 L water, ensuring enough space to maintain flooded conditions. Thirty-day-old seedlings were transplanted into pots. No NaCl was added to soils for control treatment. For 20, 40 and 60 mmol/L NaCl treatments, 15.24, 30.47 and 45.71 g pure NaCl were dissolved in 1 000 mL water and then added to the pots, respectively, at the active tillering stage (five weeks after transplanting). The soil in the pot was flooded with salt solution and the EC values of soil for control, 20, 40 and 60 mmol/L NaCl treatments were 1.00, 2.25, 5.60 and 6.58 dS/m, respectively. The crops were harvested at two weeks after salt treatment. Whole plants (with root attached) were carefully uprooted from the soil so that the root system of the plants remained unaffected.

Plant height, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight and number of tillers per hill were recorded.

Biochemical analysis

Assay of chlorophyll content

Chlorophyll content was measured according to Porra et al (1989). An aliquot of fresh leaf (0.5 g) was suspended in 10 mL of 80% acetone, mixed well and kept at room temperature in the dark for 7 d. The supernatant was collected after centrifugation at 5 000 r/min for 15 min. The sample absorbance was recorded at 645 and 663 nm using a spectrophotometer (Model 336001, Spectronic Instruments, USA).

Assay of proline content

Proline content was measured according to Bates et al (1973). An aliquot of fresh leaf (0.5 g) was