Saudi Journal of Biological Sciences (2016) **23**, 467–477

King Saud University

Saudi Journal of Biological Sciences

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ORIGINAL ARTICLE

Physiological and biochemical parameters for evaluation and clustering of rice cultivars differing in salt tolerance at seedling stage

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Received 12 February 2015; revised 12 May 2015; accepted 17 May 2015
Available online 23 May 2015

KEYWORDS

Antioxidant enzymes;
Anthocyanins;
Rice;
Salt stress

Abstract Salinity tolerance levels and physiological changes were evaluated for twelve rice cultivars, including four white rice and eight black glutinous rice cultivars, during their seedling stage in response to salinity stress at 100 mM NaCl. All the rice cultivars evaluated showed an apparent decrease in growth characteristics and chlorophyll accumulation under salinity stress. By contrast an increase in proline, hydrogen peroxide, peroxidase (POX) activity and anthocyanins were observed for all cultivars. The K^+/Na^+ ratios evaluated for all rice cultivars were noted to be highly correlated with the salinity scores thus indicating that the K^+/Na^+ ratio serves as a reliable indicator of salt stress tolerance in rice. Principal component analysis (PCA) based on physiological salt tolerance indexes could clearly distinguish rice cultivars into 4 salt tolerance clusters. Noteworthy, in comparison to the salt-sensitive ones, rice cultivars that possessed higher degrees of salt tolerance displayed more enhanced activity of catalase (CAT), a smaller increase in anthocyanin, hydrogen peroxide and proline content but a smaller drop in the K^+/Na^+ ratio and chlorophyll accumulation.

1. Introduction

Salinity is a common abiotic stress that severely limits crop growth and development, productivity and causes the continuous loss of arable land, which results in desertification in arid and semi-arid regions of the world (Pons et al., 2011). It is estimated that more than 800 million hectares of land throughout the world are adversely affected by high salinity (Munns and Tester, 2008). Saline soils are characterized by excess of

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2015.05.013
1319-562X

sodium ions with dominant anions of chloride and sulfate resulting in higher electrical conductivity (>4 dS m^{-1}) (Ali et al., 2013). In general, salinity stress induces an initial osmotic stress and subsequent toxicity as a consequence of the accumulation of ions. However, damage can also ensue as a result of excessive reactive oxygen species (ROS) such as superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\cdot}OH$) produced at a high rate commonly accumulated in plant tissues due to ion imbalance and hyperosmotic stresses. ROS accumulation leads to lipid oxidation and has a negative effect on cellular metabolism and physiology, thus adversely ruining the membrane integrity (Munns et al., 2006). Salinity tolerance in glycophytic crops including rice is predominantly associated with the maintenance of ion homeostasis, particularly low Na^+/K^+ or high K^+/Na^+ ratios, through exclusion, compartmentation, and partitioning of Na^+ (Blumwald, 2000). In addition to ion homeostasis strategies, many plants have evolved mechanisms to regulate the synthesis and accumulation of compatible solutes such as proline and glycine betaine, which function as osmoprotectants and have a crucial role in plant adaptation to osmotic stress through stabilization of the tertiary structure of proteins (Zhu, 2002; Munns and Tester, 2008).

Rice is the most important global food crop that feeds over half of the world population and more than 400 million people in rice producing areas of Asia, Africa and South America are still getting a major proportion of their energy requirement from rice and its derived products with the demand for food expected to increase by another 38% within 30 years (Surridge, 2004; Joseph et al., 2010). However, rice productivity in many areas is affected by salinity stress, which originates from the accumulation of underground salt and is exacerbated by salt mining, deforestation and irrigation (Akbar, 1986). Rice is generally characterized as a salt sensitive crop but the extent of its sensitivity varies during different growth and developmental stages. It is tolerant to salinity stress during germination and active tillering, whereas it displays more sensitivity during early vegetative and reproductive stages (Lutts et al., 1995; Zhu et al., 2001). A large body of literature is available for common white rice varieties, whereas data on the salt tolerance ability and physiological traits of highly nutritive pigmented rice are relatively scarce. Recently, black glutinous rice or pigmented rice has received increasing attention due to high amounts of bioactive properties and numerous health benefits. Evaluation and comparison of growth and physiological traits of rice genotypes subjected to salinity stress using principle component analysis (PCA) to explore relationship between physiological and biochemical parameters, are considered as one useful way for breeders to better understand physiological changes during development of rice varieties grown on salt-affected soils, which are essential for the development of rice with higher salt tolerant trait. The present study was therefore, conducted to explore growth and physiological changes in seedlings subjected to salinity stress in rice varieties differing in their level of salt tolerance along with classification of rice varieties with diverse growth and physiological parameters employing PCA. The study was conducted on rice seedlings of twelve genotypes, composed of four white and eight black glutinous rice cultivars. The information gained may be used to assist in the evaluation of relative field performance of different rice genotypes and characterization of contributing physiological traits that may be employed as reliable indicators for breeding and selection for salt tolerance.

2. Materials and methods

2.1. Plant materials, plant culture and salinity stress

Twelve rice (*Oryza sativa* L.) cultivars, including eight local black glutinous rice cultivars kindly provided by the Faculty of Agriculture of Khon Kaen University and four white rice cultivars obtained from Biotech, Thailand, were screened for their salt tolerance levels at the seedling stage. Pokkali, the salt-tolerant landrace from India, and the salt-susceptible IR29 were used as a standard check in salt screening.

For an establishment of seedlings rice seeds were surface sterilized, soaked in distilled water for 24 h and then germinated on wet filter papers embedded in Petri dishes. Subsequently three-day-old seedlings of each rice cultivar raised in Petri dishes were transplanted into pots (15 cm height × 30 cm diameter) each containing 7 kg of homogeneous mixture (11:6:3) of paddy husk, soil and farmyard manure. Fifteen seedlings were planted in each pot. After 21 days of transplanting, seedlings of each rice cultivar were subjected to salinity stress at 100 mM NaCl for 14 days. Sampling was performed at the end of the experiments and physiological changes were evaluated. Scoring of visual salt stress injury and growth reduction of rice seedlings treated with 100 mM NaCl were performed using the Standard Evaluation System of rice (SES, Table 1) (Gregorio et al., 1997). Seedling shoots and roots were separated into aerial and below ground part for fresh weight (FW) and dry weight (DW) determinations. The determination of DW was performed after drying the seedling parts in a hot-air oven at 60 °C for 3 days. Evaluations of ion concentration, proline content, total chlorophyll (TC) content, total anthocyanin (TA) content, H_2O_2 content and antioxidant enzyme activities (POX and CAT) were included in this study.

2.2. Measurement of ion concentration

Following 3 days of oven-drying of seedling shoots at 60 °C, about 0.5 g of each dried powdered sample was digested with 10 mL of nitric acid at 300 °C, 5 mL of perchloric acid at 200 °C and 20 mL of 6 M hydrochloric acid. The

Table 1 Modified standard evaluation score (SES) of visual salt injury at seedling stage (Gregorio et al., 1997).

Score	Observation	Tolerance
1	Normal growth on leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Sensitive
9	Almost all plants dead or dying	Highly sensitive