EVALUATION OF PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF RICE (ORYZA SATIVA L.) VARIETIES TO SALT STRESS

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ABSTRACT

A hydroponics study for salt tolerance was conducted in ten rice varieties, differing in salt tolerance viz., TRY 1, TRY(R)2, TRY 3 (TNAU, Coimbatore), CSR 13, CSR 27, CSR 30, CSR 36 (CSSRI, Karnal), Ezhome-1 and Ezhome-2 (KAU, Trissur) and IR 29 at seedling stage. The influence of NaCl at 0, 60 and 120 mM concentrations on seedling dry weight, shoot length, photosynthetic rate, superoxide dismutase activity, ascorbate peroxidase activity, malondialdehyde concentration and Na+/K+ ratio was investigated. Increasing salt levels reduced the photosynthetic rate, dry weight and shoot length substantially in rice varieties with greater reduction in the sensitive variety IR 29. The activities of SOD and APX were enhanced in tolerant varieties which contributed to lower lipid peroxidation. The results indicate that a low Na+/K+ ratio along with increased activity of antioxidant enzymes and lower lipid peroxidation may contribute to the NaCl tolerance mechanism in rice varieties.

Key words: Dry weight, Malondialdehyde, Na+/K+ ratio, Oryza sativa, Photosynthetic rate, Reactive oxygen species, Rice, Salinity.

INTRODUCTION

Increasing salinization of agricultural lands is one of the major challenges facing modern agriculture (Kronzucker et al., 2008). Salinity of arable land is an increasing problem of many irrigated, arid and semi-arid areas of the world where rainfall is insufficient to leach salts from the root zone, and it is a significant factor in reducing crop productivity (Francois and Maas, 1994). It has been estimated that more than half of the yield potential of major crops are usually lost due to unfavorable growing environments such as drought or high salinity (Cortina and Culiáñez-Macià, 2005). Since the opportunities to expand the arable land are narrow, the increase in agricultural productivity in normal soils as well as less productive soils is an absolute requisite to feed the world.

Rice is a major crop in Asia, providing food to more than half of the world population. Certain rice varieties have been reported as being salt sensitive at their seedling and reproductive stages leading to reduced crop productivity of more than fifty per cent (Moradi and Ismail, 2007). The most economic and sustained way to overcome the problem of salt stress in rice is the development and use of tolerant varieties.

Salinity affects several physiological pathways including photosynthesis, respiration, nitrogen fixation and carbohydrate metabolism had been observed to be affected by high salinity. In addition, it also leads to production of reactive oxygen species, which is main source of damage to cells during biotic and abiotic stress, such as super oxide anion (O\(^{-2}\)), hydrogen peroxide (H\(_2\)O\(_2\)) and the hydroxyl radicals (OH). These species of oxygen are highly cytotoxic and can seriously react with vital biomolecules such as lipids, proteins, nucleic acid, etc, causing lipid peroxidation, protein denaturing and DNA mutation respectively (Quiles and Lopez, 2004).

Many studies reported that plants possess several antioxidant enzymes like super oxide dismutases (SOD), catalases (CAT) and peroxidase (POX), and glutathion reductase (GR) to participate in the detoxification of ROS. Scientists have reported

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higher anti-oxidative capacity conferring salt tolerance in rice under salt-stress. Kumar et al. (2009) also suggested that better antioxidant machinery may be correlated with the salt tolerance ability of the tolerant rice genotypes. Evidence suggests that membranes are the primary sites of salinity injury to cells and organelles because ROS can react with unsaturated fatty acids to cause peroxidation of essential membrane lipids in plasmalemma or intracellular organelles. The most physiological parameter considered to be reliable for screening genotypes for salt tolerance is lower Na+/K+ ratio as the end result of salt responses is lowering of Na+ concentration in the actively growing functional tissues (Gregorio and Senadhira 1993).

MATERIALS AND METHODS

Salinity treatments and rice varieties

The experiment was carried out in a randomized completely block design with three replications and three treatments i.e. a control (without stress), 60 mM NaCl and 120 mM NaCl. Ten different rice varieties varying in salt tolerance abilities collected from three different geographical locations included TRY 1, TRY(R)2, TRY 3 (TNAU, Coimbatore); CSR 13, CSR 27, CSR 30 and CSR 36 (CSSRI, Karnal) and Ezhome-1 and Ezhome-2 (KAU, Trissur) along with IR 29 (salt sensitive) were used for the study.

The rice seeds treated with 1 % Mercuric chloride solution, and were soaked in distilled water over-night. After 5-6 days of sowing, the seedlings were transferred to plastic trays containing Modified Yoshida solution (Fig 8). The pH of the solution was maintained at 4.5 constantly. The solution was changed once in seven days. Salt stress was applied three weeks after sowing in the Modified Yoshida solution, using sodium chloride.

Measurement of growth and photosynthetic rate

Shoot length and dry weight were measured after fourteen days of salinization. The gas exchange parameters viz., photosynthetic rate (µmol CO₂ m⁻² s⁻¹) was recorded on the fully expanded leaves in the plants seven days after imposing salt stress, using LI-COR (LI-6400XT) Portable Photosynthesis System (PPS) at 10:00 h (IST). While taking measurements, the photosynthetically active radiation of 1500 µmol m⁻² s⁻¹ was maintained with the in-built light source, temperature was 25±5°C, relative humidity was 65±5 % and reference carbon dioxide concentration was 380 mol CO₂ mol air⁻¹.

Super oxide dismutase (SOD) and Ascorbate peroxidase (APX) assay

The assay was conducted as per the procedure by Beauhamp and Fridovich (1971). The ascorbate peroxidase activity (APX) was determined by modifying the procedure of Nakano and Asada (1981).

Lipid Peroxidation

Malondialdehyde was measured by method of Stewart and Bewley (1980). 0.5 g of leaf samples were homogenized in 5 ml of distilled water. An equal volume of 0.5 % (w/v) thiobarbituric acid (TBA) in 20 % (w/v) trichloroacetic acid solution was added and the sample incubated at 95°C for 30 min. The reaction stopped by putting the reaction tubes in the ice bath. The samples then centrifuged at 10000 rpm for 30 min. The supernatant was collected and absorption was read at 532 nm. The amount of non-specific absorption at 600 nm was subtracted from this value. The amount of MDA present calculated from the extinction coefficient of 155 mM⁻¹cm⁻¹.

Na+/K+ ratio

The Na+/K+ ratio of the shoot samples of the treated and control plants were analyzed using Flame Photometer (ELICO, Model CL-361), following triple acid (HNO₃, H₂SO₄ and HClO₄ in the ratio of 9:2:1) digestion of the samples (Jackson, 1967).

RESULTS AND DISCUSSION

Salinity stress negatively affects the physiological and biochemical processes in the plant development. The present study demonstrated a significant variation among rice cultivars in physiological and biochemical responses to salinity stress. Comparison of varietal differences in rice plants will be extremely useful in studying the mechanism of resistance. The varietal difference is recognized with respect to various parameters such as growth in length and weight, survival and physiological features in addition to ion exclusion capacity (Noble and Rogers, 1992).

Growth reduction was observed in plants exposed to different level of salinity stress. Shoot length of all genotypes was reduced in both the salt stress treatments (Fig.1). The per cent reduction of
shoot length under 60 mM NaCl concentration was lowest in CSR 36 (5.02) and maximum in IR 29 (19.47). Under 120 mM NaCl, Ezhome-2 recorded the lowest reduction of 19.30 per cent and CSR 13 recorded the maximum of 32.12 per cent. The salt tolerant cultivars maintained a better shoot length compared to the susceptible variety IR 29 which recorded the lowest shoot length in all the treatments (Fig. 1). This is in accordance with Lee et al. (2003) who reported a growth reduction in sensitive varieties of japonica and indica rice seedlings exposed to salinity stress. The decrease in the shoot and root growth in salinized plants could be attributed to several reasons. Among them, most important is the reduction in photosynthesis that limits the supply of carbohydrates needed for growth. Secondly, reduction in turgor in expanding tissues resulting from lowered water potential in root growth medium. Salinity causes pronounced effect on fresh and dry weights in salt sensitive while salt tolerant cultivars were able to maintain its fresh and dry weights (Pattanagul and Thitisaksaul, 2008). In the present study the dry weight of the varieties were significantly reduced by salt stress (Fig 2). The per cent reduction in dry weight was comparatively lower for CSR 27 (19.87 and 43.31) under both 60 mM and 120 mM NaCl concentrations respectively. The observed reduction in plant biomass might be due to a combination of slower growth and development as a result of osmotic stress, inhibition of photosynthesis as a result of direct effects of salinity on the photosynthetic apparatus or indirect effects as a result of reduction in sink capacity (Kato and Takeda, 1996). The important of photosynthesis under salinity is further strengthened by a positive correlation between photosynthetic rate and dry weight (Fig. 3).

The reduction in photosynthesis and growth are the most conspicuous effects of salinity stress. The photosynthetic rate of the genotype IR 29 was reduced by 32.97 per cent under 60 mM NaCl treatment while CSR 27 recorded a reduction of 16.29 per cent. Under 120 mM NaCl, CSR 27 and Ezhome-2 recorded the highest photosynthetic rate, where as the photosynthetic rate of IR 29 was reduced by 61.08 per cent. Though various studies have shown negative effect of salinity on photosynthesis using soil level salinity, the present study demonstrated in hydrophonic system where the direct effect has been observed.

Production of reactive oxygen species increased under saline conditions and reactive oxygen species mediated membrane damage has been demonstrated to be a major cause of the cellular toxicity by salinity in rice, tomato and citrus (Mittova et al., 2004). Super oxide dismutase activity was observed to vary with varieties, but CSR 27 reported a higher activity followed by Ezhome-2 irrespective of the salt treatments (Fig. 5). At higher salt concentration of 120 mM NaCl, the super oxide dismutase activity of the genotype IR 29 was found to be decreased. It has been shown that salinity increases SOD activity in salt-tolerant cultivars and decreases the activity in salt-sensitive cultivars, in both leaves (Hernandez et al., 2000) and roots (Shalata et al., 2001). The reduced activity of SOD in sensitive cultivar can affect the ability of the seedlings to scavenge free radicals, which in turn cause membrane damage.

Ascorbate peroxidase activity was found to increase with the increase in salt concentration in almost all varieties. The varieties TRY 1 and Ezhome-1 recorded the highest activity under 60 mM NaCl where as the varieties TRY 3 and CSR 13 recorded the highest activity under 120 mM NaCl (Fig. 6). The susceptible genotype IR 29 recorded the lowest activity. The results obtained in the present experiments are in accordance with many studies which suggested that the resistance to salt stress is correlated with a more efficient anti-oxidative system, with high enzymatic activities and better biomass production in salt tolerant cultivars of rice than sensitive ones.

Malondialdehyde, a compound produced by the lipid peroxidation of the cell membrane is often used as an indicator of salt and oxidative damages (Mandhania et al., 2006). It has widely been utilized to differentiate salt tolerant and salt sensitive cultivars (Xue and Liu, 2008). The salt sensitive genotype IR 29 reported the highest lipid peroxidation under both the levels of salinity. In the presence of salt, malondialdehyde content was least in salt tolerant cultivars. The salt tolerant variety CSR 27 followed by Ezhome-2 recorded the lowest lipid peroxidation in both 60 mM) treatments (153.50 and 156.38) and 120 mM NaCl (182.81 and 198.23), but with an
FIG 1. Impact of salinity on shoot length in different rice varieties.

FIG 2. Impact of salinity on dry weight in different rice varieties.
FIG 3. Correlation between photosynthetic rate (µ mole of CO₂ m⁻² s⁻¹) and dry weight under salinity.

FIG 4. Impact of salinity on MDA (µ mole g) in different rice varieties.
FIG 5. Impact of salinity on SOD activity (Units mg\(^{-1}\) protein) in different rice varieties.

FIG 6. Impact of salinity on APX (Units mg\(^{-1}\) protein) in different rice varieties.
increase in lipid peroxidation as the salt concentration increased (Fig. 4). This is in accordance with the results of Sairam and Srivastava (2002) who reported the enhancement of lipid peroxidation and membrane damage in the leaves subjected to higher degree of salt stress than mild stress.

The negative effect of sodium on plant physiological and biochemical processes has been shown in different crops. The shoot Na⁺/K⁺ ratio is considered to be a reliable parameter used to evaluate the salt tolerance ability of rice cultivars (Greagario et al., 1997). The high sodium accumulation in salt sensitive foxtail millet cultivar, tomato roots and rice roots resulted in an increased membrane damage, and oxidative damage (Mandhana et al. 2006). The salt sensitive genotype IR 29 has a higher shoot sodium content which resulted in the oxidative damage. The present results clearly showed that the salt tolerant varieties recorded lower Na⁺/K⁺ ratio whereas the susceptible variety IR 29 recorded the highest Na⁺/K⁺ ratio. The lowest Na⁺/K⁺ ratio was recorded by Ezhome-2 and CSR 27. The low Na⁺/K⁺ ratio at seedling stage are associated with tolerance and possible reason for this can be Na⁺ exclusion as well as partitioning of Na⁺ into older leaves as reported by Ren et al. (2005).
Plate 8. Rice varieties after fourteen days of salinization

FIG 8. Experimental set up showing phenotypic differences among the rice varieties under salinity.
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