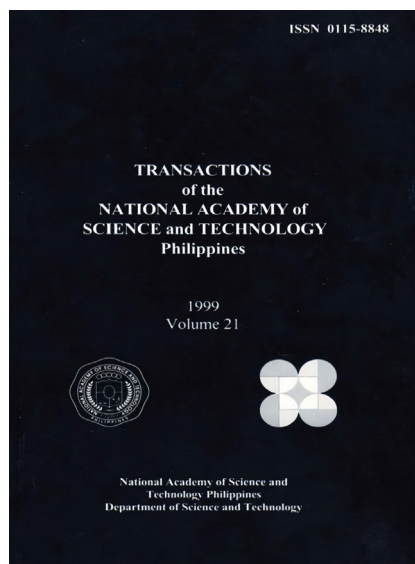


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Varietal Differences in Growth, Sodium Uptake and Antioxidant Responses of Rice Seedling to Salinity Stress

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Citation

Dionisio-Sese ML. 1999. Varietal differences in growth, sodium uptake and antioxidant responses of rice seedling to salinity stress. Transactions NAST PHL 21: 216-226. doi.org/10.57043/transnastphl.1999.5767

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VARIETAL DIFFERENCES IN GROWTH, SODIUM UPTAKE AND ANTIOXIDANT RESPONSES OF RICE SEEDLING TO SALINITY STRESS

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ABSTRACT

The growth rate, leaf sodium uptake and possible involvement of activated oxygen species in the mechanism of damage by NaCl stress was studied in four varieties of rice (*Oryza sativa L.*) exhibiting different sensitivities to NaCl. The 3-week-old rice seedlings were subjected to 0, 6 and 12 dS m⁻¹ (equivalent to 0, 60 and 120 mM NaCl) salinity levels for 1-week after which antioxidant capacities, growth rate and Na⁺ uptake of the leaves were analyzed. High salinity treatment caused a decrease in growth rate in all the varieties tested except Pokkali. Varieties that are considered to be salt-sensitive, Hitomebore, IR28 and Bankat, exhibited a decrease in superoxide dismutase activity and an increase in peroxidase activity under high salinization. These varieties also exhibited increase in lipid peroxidation and electrolyte leakage as well as higher Na⁺ accumulation in the leaves under salt stress. The salt-tolerant variety Pokkali, however, showed only slight increase and decrease in superoxide dismutase and peroxidase activity, respectively, and virtually unchanged lipid peroxidation, electrolyte leakage and Na⁺ accumulation upon salinization. These results indicate that free radical-mediated damage of membrane may play an important role in the cellular toxicity of NaCl in rice seedlings and that salt-tolerant varieties exhibit protection mechanism against increased radical production by maintaining the specific activity of antioxidant enzymes.

Keywords: antioxidant responses, electrolyte leakage, growth rate, lipid peroxidation, *Oryza sativa L.*, oxidative stress, peroxidase, salt stress, sodium chloride stress, superoxide dismutase

INTRODUCTION

Soil salinity primarily due to Na salts, particularly NaCl, is a major stress factor reducing plant growth and productivity throughout the world. The rapid expansion in the use of irrigation to meet the increasing demand for food crops and plant products has led to a decrease in crop productivity as a result of salinity stress.

Although most cereal crops exhibit high tolerance to soil salinity, a notable exception, however, is rice, the staple food of the majority of the world's population. Due to this, great interest focused on screening and developing rice varieties that are tolerant to salinity stress. Defining salt tolerance, however, is quite difficult because of the complex nature of salt stress and the wide range of plant responses. Thus, a better understanding of the mechanism that enable plants to adapt to salinity stress and maintain growth, development, and productivity during stress periods could help in breeding and genetic engineering of crop plants for salinity resistance.

Present evidences suggest that many damaging environmental stresses have their effects directly or indirectly through the formation of activated oxygen species following impairment of electron transport systems. These activated oxygen species, such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\bullet OH$) and singlet oxygen (1O_2) have the potential to inactivate enzymes and damage important cellular components such as lipids, proteins and nucleic acids (Davies 1987, Fridovich 1986). Fortunately, plants have evolved various enzymatic and non-enzymatic mechanisms that can minimize the damaging effects of activated oxygen. Among the enzymatic antioxidants are superoxide dismutase (SOD), catalase, peroxidase and enzymes of the ascorbate-glutathione cycle.

This study was designed to determine the effect of salt stress on growth, Na^+ content, antioxidant enzyme activities, lipid membrane peroxidation and electrolyte leakage of leaves of rice varieties exhibiting differences in salinity tolerance. Comparison of these responses could be useful in identifying differences related to the relative ability of each cultivar to cope with salinity. Result from this study can supply information on the involvement of activated oxygen species in the mechanism of damage by NaCl stress in rice plant, and also could allow deeper insights into the molecular mechanisms of tolerance to salt-induced oxidative stress.

MATERIALS AND METHODS

Plant materials and salinity treatments

Four rice varieties differing in salt tolerance and categorized into two ecogeographic landraces were used. The japonica landraces were Hitomebore (salt-sensitive) and Bankat (salt-sensitive) while the indica landraces comprised of IR28 (salt-sensitive) and Pokkali (salt-tolerant). Seeds of each variety were sown on styrofoam boards floating in deionized water and placed in a glasshouse under natural day and night light temperature maintained at 28°C. After one week,

deionized water was replaced with one-fourth strength modified Yoshida nutrient solution (Mae 1993) adjusted to pH 5.8 with 1 N KOH. After another week, the nutrient solution was replaced with one half strength nutrient solution and renewed twice a week. Water lost by evapotranspiration was compensated for by the daily addition of deionized water. Three weeks after sowing salinization was induced by adding NaCl to the one-half strength modified Yoshida solution to obtain electrical conductivities of 6 and 12 dS m⁻¹, which are equivalent to about 60 and 120 mM NaCl, respectively. Nutrient solution without NaCl addition (0 mM NaCl or 0 dS m⁻¹) served as the control.

Measurements were taken one week after salinity treatments on a completely randomized design with four replicates.

Growth measurements

Plant growth was estimated through relative growth rate (RGR) measurement. This was calculated from the increase in dry weight of plant shoots at the beginning and at the end of the salt treatment, using the equation $RGR = (\ln W_7 - \ln W_0)/(t_7 - t_0)$ where W is the shoot dry weight, t is the time and subscripts denote sampling 0 and 7 days after salinity treatment.

Sodium determination

The sodium content of the leaf, which represents plant sodium uptake, was determined by atomic absorption spectrophotometry on cooled extract of dried cut material in distilled deionized water, heated in a boiling water bath for one hour, and then autoclaved at 121°C for 20 minutes.

Determination of enzyme activities and lipid peroxidation

Assay of enzyme activities and estimation of lipid peroxidation were done on 50 mM phosphate buffer extracts (pH 7.0) of the frozen leaf samples. Total SOD activity, the basis of which is its ability to inhibit the photochemical reduction of nitro blue tetrazolium, and peroxidase activity using the guaiacol oxidation method were determined as described previously (Dionisio-Sese and Tobita 1998). Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) formation using the thiobarbituric acid method described by Stewart and Bewley (1980).

Electrolyte leakage measurement

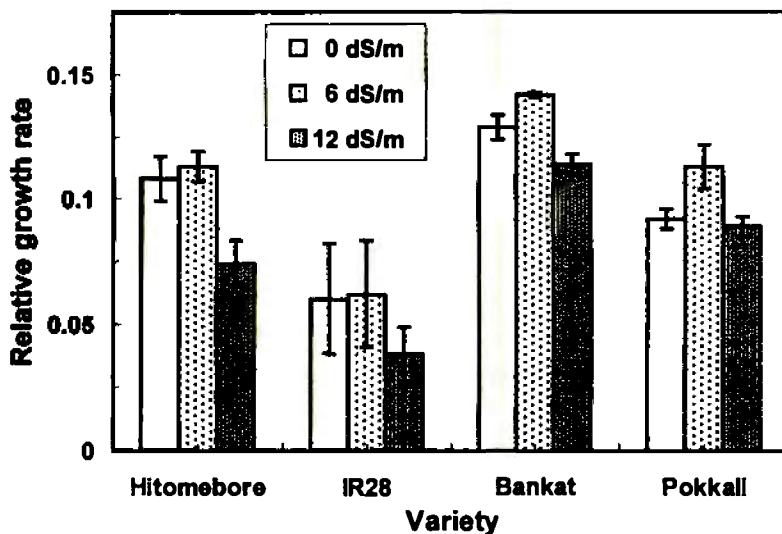
The electrolyte leakage rate (EL) was expressed following the formula $EL = EC_1/EC_2 \times 100$ where EC_1 and EC_2 , respectively, are the electrical conductivity measurements of fresh leaf samples placed in water bath at 32°C for two hours and after autoclaving at 121°C for 20 minutes.

RESULTS AND DISCUSSION

There has been considerable effort directed at selection and development of rice varieties resistant to salinity stress. Progress, however, seems slow primarily due to an inadequate knowledge of salt tolerance mechanism.

In rice, as well as in any other crops, agronomic characters, such as yield, survival, leaf damage and plant height have been the most commonly used criteria for identifying tolerance (Lutts *et al.*, 1995, Yeo *et al.*, 1990). This is largely due to measurement ease and yield under saline conditions which is usually the ultimate requirement. In the present experiment, on the basis of the shoot's dry weight measurement during one-week salt treatment at the seedling stage, varieties Hitomebore, IR28 and Bankat, which are considered salt-sensitive, showed growth retardation at 12 dS m⁻¹ (Fig. 1). In contrast, the salt-tolerant variety, Pokkali, did not show any growth inhibition at the same salinity level. Salinization was induced at the seedling stage since previous studies showed that during the vegetative growth of rice, this stage is the most sensitive to NaCl compared to germination and tillering stages (Lutts *et al.*, 1995).

It has been proposed, however, that selection and breeding to increase salt tolerance might be more successful if based directly, not on agronomic characters, but on the physiological mechanisms conferring tolerance (Noble and Rogers,



1992). Since salinity has both osmotic and ion effects on plant growth, ion exclusion is being considered as a selection criterion for improving salt tolerance in crops (Noble and Rogers, 1992). Rice plant studies revealed that the cause of injury from salinity stress is more likely to be from excessive sodium and not chloride ion. This is because Cl^- is tolerated over a wide range of concentrations (Clarkson and Hanson, 1980), and the disruptive effect of Na^+ in the conformation of macromolecular structure and its interference with the roles of cytoplasmic K^+ will preempt Cl^- toxicity (Gregorio and Senadhira, 1990).

Results of the analysis of Na^+ content of leaves of rice varieties differing in salt tolerance agree with the view that there is an inverse relationship between shoot Na concentration and salt tolerance (Yeo and Flowers, 1983). The salt-tolerant variety Pokkali did not exhibit a significant increase in Na^+ accumulation in the leaves even at high salinity level whereas the salt-sensitive varieties Hitomebore, IR28 and Bankat showed pronounced accumulation (Fig. 2).

It has been observed that when plants are subjected to environmental stress such as drought (Dhindsa and Matowe 1981), chilling (Wise and Naylor 1987), high light intensity and mineral deficiency (Cakmak and Marschner, 1992), ultraviolet radiation (Rao and Omrod, 1995), and herbicide treatment (Harper and Harvey 1978), the balance between the production of activated oxygen species and the quenching activity of antioxidants is upset, often resulting in oxidative damage. A greater resistance to this oxidative damage was observed in plants

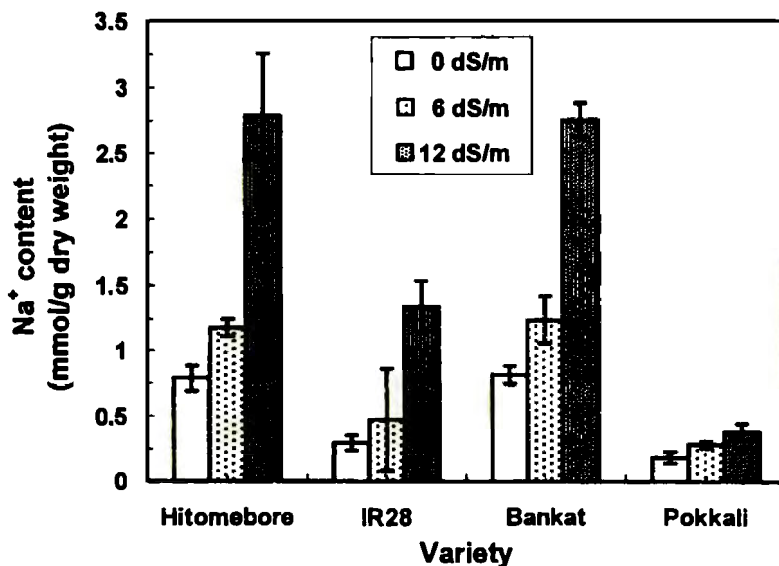


Figure 2. Effect of increasing salinity on Na^+ accumulation in the leaves of four rice varieties.

having high levels of antioxidants (Harper and Harvey 1978, Dhindsa and Matowe 1981, Wise and Naylor 1987).

The present study indicates different responses in the activities of antioxidant enzymes involved in the oxygen metabolism, and that these responses might be related to the distinct susceptibilities of the rice varieties to NaCl. Increasing magnitude of salinity stress brought about a significant decrease in SOD activity in the salt-sensitive varieties whereas the salt-tolerant variety Pokkali did not show any decline in SOD activity at all – in fact a slight increase in SOD activity was observed in Pokkali with increasing salinization (Fig. 3). Singha and Choudhuri (1990) also observed that NaCl decreased SOD activity in rice seedlings. Since no comparison was done among varieties differing in salt tolerance, it is assumed that the variety they have used for analysis was of the salt-sensitive type.

Aside from rice, salinity induced the decrease in SOD activity in the leaves, chloroplasts and mitochondria of pea plants (Hernandez *et al.* 1993; 1995). This decrease in SOD activity may be due to either decrease of enzyme synthesis, increase of enzyme degradation, or alternatively to conformational changes and reduced structural stability of the enzyme molecule. Hernandez *et al.* (1994) demonstrated in cowpea plants that the catalytic activity of SOD isozymes decreased as a function of salt concentration *in vitro*. Although the compartmentation of Na⁺ within leaves and inside the cells was not determined in the present study, the high ion content and the parallel decrease in SOD activities found in the leaves of

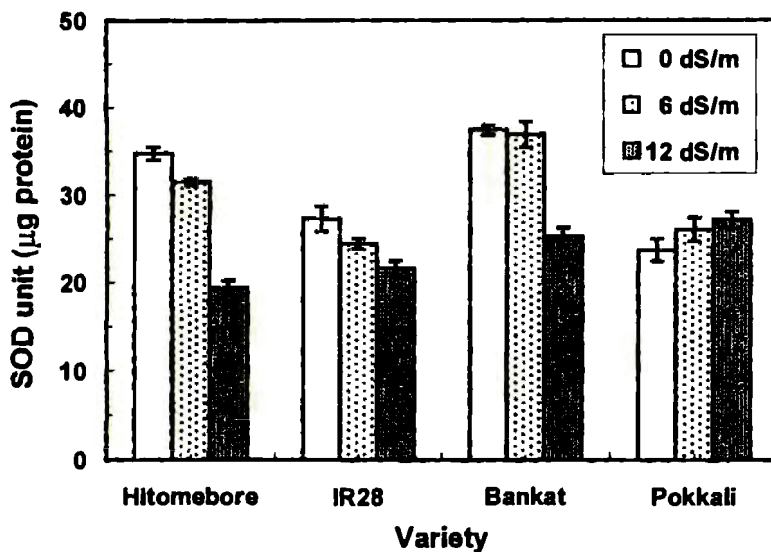


Figure 3. Effect of increasing salinity on the activity of SOD in the leaves of four rice varieties.

salinized rice plants is consistent with the possibility that salt directly inhibits the catalysis *in vivo*. However, the other possibility that the inhibition of SOD activity under salt stress is a consequence of an altered synthesis and accumulation of less active enzyme in salt-treated plants cannot be ruled out. Thus, there is a need for more data concerning how salinity stress may affect SOD activity, either directly on catalysis or through total enzyme capacity.

Since SOD is a major scavenger of $O_2^{\bullet-}$, the observed decrease in SOD activity in salinized salt-sensitive rice varieties, in turn, could favour an accumulation of $O_2^{\bullet-}$ radicals causing membrane damage. The extent of damage to the membrane can be monitored by measuring the amount of MDA produced when polyunsaturated fatty acids in the membrane undergo peroxidation. Unlike the salt-tolerant Pokkali, there was an observed increase of MDA in all the varieties tested when exposed to NaCl stress (Fig. 4). By generating changes in unsaturated fatty acids that affect membrane structure and properties, this enhanced free radical formation and lipid peroxidation under salt stress may also bring about an increase in membrane permeability or loss of membrane integrity. This is proven by the increase in electrolyte leakage in salt-sensitive varieties with increasing magnitude of salinity stress whereas the rate of electrolyte leakage was almost unchanged in Pokkali (Fig. 5). Salt stress-induced electrolyte leakage has also been previously observed in tomatoes (Tal and Shannon, 1983) and melons (Borochoy-Neori *et al.*, 1991).

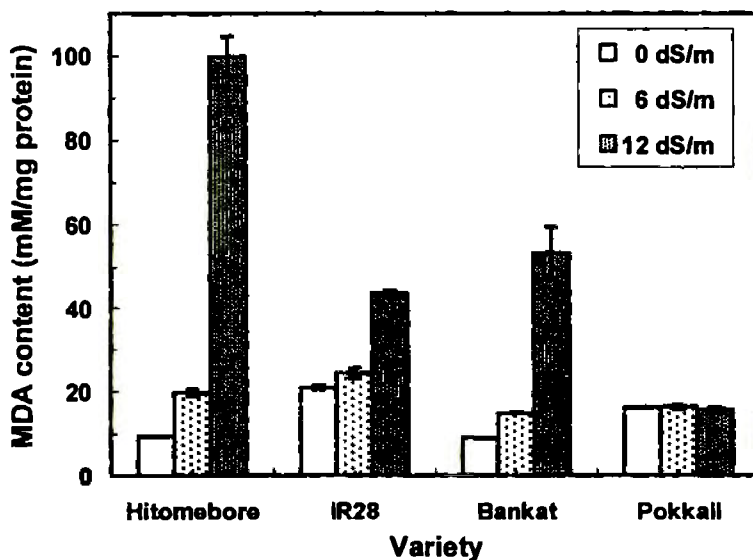


Figure 4. Effect of increasing salinity on MDA content in the leaves of four rice varieties.

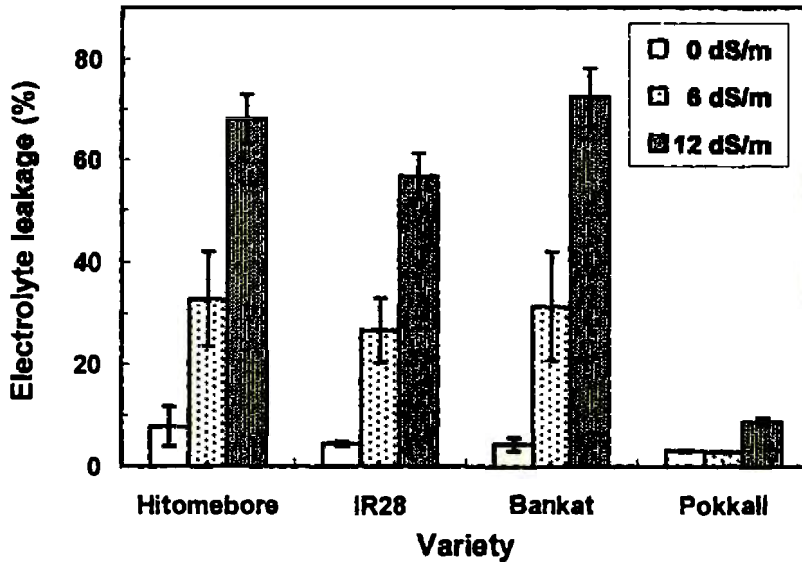


Figure 5. Effect of increasing salinity on electrolyte leakage rate of the leaves of four rice varieties.

Unlike SOD, peroxidase activity increased with increasing salinity level in Hitomebore, IR28 and Bankat whereas a slight decrease was observed in the tolerant Pokkali upon increasing exposure to salinity stress (Fig. 6). Various researchers dealing with rice (Mittal and Dubey, 1991) and other plants (Sheoran and Garg, 1979; Kalir *et al.*, 1984) have also reported increase in peroxidase activity under salt-stress. It is not clear whether the observed increase in peroxidase activity under salt stress was due to increased activity of peroxidase encoding genes or an increased activation of already existing enzymes. Mittal and Dubey (1991) suggested that salinity affects mainly the *de novo* synthesis of the enzyme since inhibition under *in vitro* conditions and activation under *in vivo* conditions was observed in salt-sensitive cultivars. Lopez *et al.* (1996), however, have shown that the salt-induced increase in ascorbate peroxidase activity in radish plants was not accompanied by a corresponding increase in mRNA level, suggesting that the salt-induced ascorbate peroxidase expression is probably the consequence of post-transcriptional events. In *Halimione portulacoides*, a halophyte which survives harsh saline conditions in salt marshes, it was suggested that the increase in peroxidase activity induced by exposure to salinity was due to conformational changes occurring in the protein molecule rather than an increase in protein synthesis or proteolysis (Kalir *et al.*, 1984).

Aside from their function in the metabolism of active oxygen, peroxidases in plants are also involved in the biosynthesis of cell wall (Negrel and Lherminier

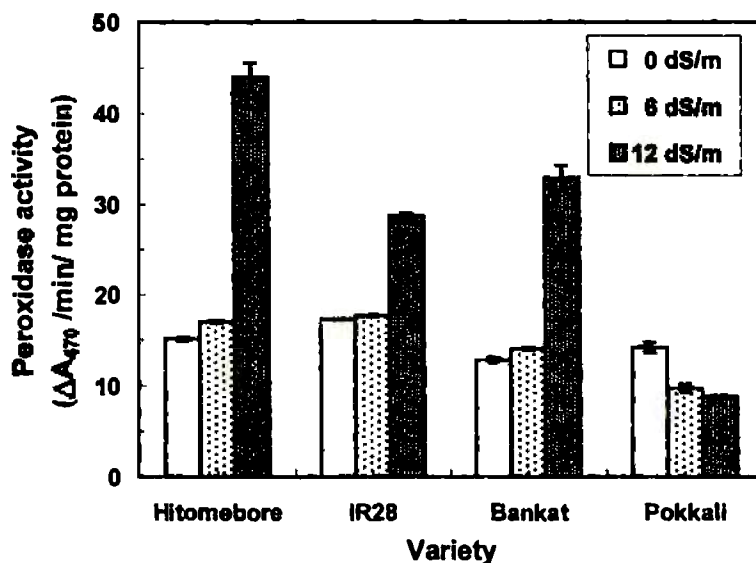


Figure 6. Effect of increasing salinity on the activity of peroxidase in the leaves of four rice varieties.

1987) including lignification and suberization (Espelie *et al.* 1986; Polle *et al.* 1994). Considerable evidence shows that high peroxidase activity is correlated with the reduction of plant growth (MacAdam *et al.* 1992; Zheng and Van Huystee, 1992, Lee and Lin, 1995). This might be attributed to peroxidase catalysis of ferulic acid conversion to diferulic acid on polysaccharides, the feruloylation of hemicelluloses, or the insolubilization of hydroxyproline-rich glycoprotein causing cell wall stiffening (Fry 1986; Waffenschmidt *et al.*, 1993). Morphologically, the most typical symptom of saline injury to a plant is retarded growth due to inhibition of cell elongation (Nieman, 1965), resulting in a stunted plant. Notwithstanding the other physiological and biochemical mechanisms involved, the observed decrease in rice growth of salt-sensitive varieties with increasing salinization might then be partly due to salt-induced increases in peroxidase activity.

The results of this study show that there were substantial differences between the growth and antioxidant responses of the four rice varieties to salinity treatment. During salt stress the salt-sensitive varieties, Hitomebore, IR28 and Bankat, exhibited high leaf Na^+ accumulation resulting in symptoms of oxidative damage such as decrease in SOD activity, increase in lipid peroxidation and electrolyte leakage, increase in peroxidase activity and decrease in growth rate. Salinity, however, only had a minimal effect on growth rate, leaf Na^+ accumulation and antioxidant metabolism in the salt-tolerant variety, Pokkali. Thus under salt stress,

the lower Na⁺ accumulation and relatively unchanged SOD and peroxidase activities, by bringing about an unchanged capacity for oxygen radical scavenging and maintenance of cellular membranes as well as cell wall function, could explain the NaCl tolerance of tolerant rice varieties over the sensitive ones.

ACKNOWLEDGEMENTS

This work was undertaken when the author was granted a Visiting Research Fellowship at the International Collaboration Research Section, Okinawa Subtropical Station, Japan International Research Center for Agricultural Sciences, Ministry of Agriculture, Forestry and Fisheries, Japan. Special thanks are extended to Dr. Satoshi Tobita for the constructive help and critical discussion and to Dr. Takaharu Hayashi and Dr. Shigeo Yashima for their managerial support.

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