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PHOTOSYNTHETIC CHARACTERISTICS AND ACTIVITY OF ANTIOXIDANT ENZYMES IN SALINITY TOLERANT AND SENSITIVE RICE CULTIVARS

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SUMMARY

A study was conducted to determine the physiological and biochemical characteristics of salinity tolerant (CSR-13) and sensitive (MI-48) rice genotypes in comparison with Pokkali (salinity tolerant check). Twenty-five days old seedlings were transplanted in earthen pots with control and two levels of salinity stress (6.17 and 11.68 dS m^{-1}) treatments. Observations were recorded for rate of photosynthesis, chlorophyll content, relative water content, lipid peroxidation and activities of RuBP Carboxylase (RuBPC), superoxide dismutase (SOD) ascorbate peroxidase (APX) and catalase (CAT) enzymes at 30 and 60 days after transplanting. Rate of photosynthesis, RuBPC activity and chlorophyll content decreased marginally by salinity stress in CSR-13 and Pokkali but MI-48 showed greater reduction. Pokkali and CSR-13 maintained higher RWC under salt stress compared to salt sensitive MI-48. Membrane lipid peroxidation was higher in both CSR-13 and MI-48 genotypes. SOD and APX activities were highly induced under salt stress in Pokkali but CAT activity decreased. In CSR-13, induction of SOD, APX and CAT activities was higher than that of MI-48. The results indicate that maintenance of higher RWC and induction of SOD and APX activities under salt stress in CSR-13 contributed to its salt tolerant characteristics.

***Key words*: Antioxidant enzymes, photosynthesis, rice, salinity**

INTRODUCTION

Soil salinity is a major constraint limiting agricultural productivity in nearly 20% of cultivated area and half of the irrigated area world wide (Zhu 2001). Salinity-induced effects on plants may be attributed to non-availability of water due to reduction in osmotic potential of soil solution, ion toxicity and nutrient imbalance/deficiency (Hasegawa *et al.* 2000). Salt stress has been reported to cause inhibition of growth and development, reduction in photosynthesis, respiration and protein synthesis in sensitive species (Boyer 1982). An important consequence of salinity stress in plants is the excessive generation of reactive oxygen species (ROS) such as superoxide anion (O^{-}_{2}), hydrogen peroxide (H_2O_2) and the hydroxyl radicals ($OH^{\bullet}$) particularly in chloroplast and mitochondria (Mittler 2002). Plants possess a number of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and ascobate peroxidases (APX) to protect against the damaging effect of ROS (Asada 1992). Salinity induced changes in activities of various antioxidant enzymes have been reported by various workers (Hernandez *et al.* 2000). Various physiological parameters have been associated with salt tolerance in crop plants. Changes in relative water content and ion exclusion are considered relevant criteria for improving drought/salt tolerance (Noble and Rogers 1992).

Because of the inherent sensitivity of rice plants to salt stress, the salinity has become a serious production constraint for rice (Francois and Mass 1994). The yield

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level can be consolidated by developing high yielding varieties with high level of tolerance to salinity stress. Considerable efforts have been made in selection and development of rice varieties tolerant to salinity stress, however, progress seems to be slow primarily due to inadequate understanding of the mechanism of salt tolerance. The present investigation therefore, attempts to analyse the physiological and biochemical basis of salt tolerance in rice genotypes differing in their sensitivity to salinity stress. Such an understanding will be useful in future breeding programme for developing salt tolerant rice genotypes.

MATERIALS AND METHODS

Twenty-five days old seedlings of three rice genotypes, viz. CSR-13 (salt tolerant), MI-48 (salt sensitive) and Pokkali (salinity tolerant check) were transplanted in earthen pots (35 × 40 cm) lined with double layers of polythene sheet filled with sandy loam soil. Before transplanting, pots were irrigated with water (control) and 50 and 100 mM NaCl solutions to give two salinity stress treatments. The salinity levels were determined in soil samples collected weekly and mean values expressed as dS m^{-1} were 0.82 (S_0), 6.17 (S_1) and 11.86 (S_2). Observations for rate of photosynthesis, chlorophyll content, relative water content, lipid peroxidation and activity of enzymes were determined at 30 and 60 days after planting (DAP).

The rate of photosynthesis and stomatal conductance were measured in the top most fully expanded leaves using portable photosynthesis system (Model Li-6200, LI-COR Inc. Nebaraska, USA). Chlorophyll was extracted by non maceration technique of Hiscox and Israelstam (1979) using dimethyl sulphoxide (DMSO) and chlorophyll contents were determined spectrophotometrically following Arnon (1949).

RuBP carboxylase was extracted following the procedure of Servaites *et al.* (1984). The enzyme activities were estimated by RuBP-dependent incorporation of $^{14}CO_2$ into acid stable product. The activity of enzyme was measured at 25 °C by injecting 100 µl of 2.5 mM RuBP and 50 µl of soluble leaf extract into an assay mixture containing (final concentrations) 50 mM Tris-HCl (pH 8.0), 20mM $MgCl_2$, 0.1% (m/v) bovine serum albumin, 20 mM $NaH^{14}CO_3$ (74 kBq per assay) in a total volume of 0.5 ml. The reaction was terminated after 60 s by addition of 200 µl of 6 M acetic acid; the reaction material was dried at 65°C and acid stable ^{14}C was estimated using liquid scintillation counter.

Leaf relative water content (RWC) was estimated following the method of Weatherley (1950). Leaf samples (0.2 g) were saturated in 100 ml of water for 4 h and the turgid weight of leaf samples was recorded. Afterwards the samples were oven dried at 60°C for one week to measure the dry weight.

Lipid peroxidation was measured by estimating concentration of TBARS (thiobarbituric acid reactive substances), a product of lipid peroxidation following the method of Heath and Packer (1968). SOD activity was estimated based on reduction in absorbance of nitro-blue tetrazolium (NBT) by enzyme following the method of Dhindsa *et al.* (1981). The catalase activity was assayed according to the procedure of Aebi (1984) by monitoring the reduction in absorbance at 240 nm as H_2O_2 was oxidized in the reaction mixture consisting of 50 mM potassium phosphate buffer, 10 mM H_2O_2 and the crude enzyme extract. APX activity was estimated, based on the reduction in absorbance at 290 nm as ascorbate was oxidized according to the method described by Nakano and Asada (1981). The reduction in absorbance was recorded 30 seconds after this addition using UV-VIS spectrophotometer (ECIL, India).

Statistical analysis of the data was done by analysis of variance (ANOVA) following Panse and Sukhatme (1967). The critical difference (CD) values between control and each treatment were calculated at 5% probability level ($P \leq 0.05$).

RESULTS AND DISCUSSION

In the present study a decreasing trend in P_N in all the three rice cultivars was observed under salinity stress. MI-48 exhibited maximum reduction at both the levels of stress (Table 1). The reduction in P_N in Pokkali was not significant at both the levels of stress, whereas, in CSR-13, the reduction in P_N was significant only at S_2 stress level. A decrease in photosynthetic carbon assimilation has also been reported in chickpea at high NaCl stress

Table 1. Rate of photosynthesis, RuBP carboxylase activity, chlorophyll content, RWC and lipid peroxidation in three rice genotypes under salt stress at 30 and 60 days after planting (DAP). S_0= Control, S_1 = 6 dS m^{-1} and S_2 = 12 dS m^{-1}, * indicates the significant difference between control and treatment (P>0.05).

Parameters	Salt Treatment	Pokkali		CSR-13		MI-48	
		30DAP	60DAP	30DAP	60DAP	30DAP	60DAP
Rate of Photosynthesis (μmol $CO_2 m^{-2} s^{-1}$)	S_0	14.45	15.26	16.78	16.22	17.05	16.65
	S_1	13.88	13.97	15.02	15.08	13.22*	12.95*
	S_2	12.75	13.02	12.41*	12.96*	10.81*	11.07*
Rubisco activity (μmol CO_2 g^{-1} fw min^{-1})	S_0	23.14	25.23	26.45	28.05	22.36	25.72
	S_1	20.08	22.97	21.88	24.81*	18.39	17.95*
	S_2	18.46*	19.02*	16.54*	20.17*	15.62*	16.38*
Chlorophyll content (mg g^{-1} fw)	S_0	2.41	2.04	2.62	2.27	2.17	2.35
	S_1	2.48	1.96	2.46*	2.21	1.94*	2.12*
	S_2	2.12	1.85*	2.13*	2.02*	1.75*	1.96*
Relative water content (%)	S_0	76.9	80.5	77.2	75.5	74.6	75.3
	S_1	77.4	76.2	74.6	74.6	68.1*	67.4*
	S_2	71.5	73.4*	71.3*	70.5	62.5*	63.2*
TBARS content (nmol g^{-1} dw)	S_0	214.25	238.46	298.54	317.18	314.0	302.47
	S_1	257.56	281.21*	573.00*	602.45*	698.2*	644.50*
	S_2	427.51*	455.32*	894.50*	981.56*	1256.5*	1177.20*

resulting from inhibition of rubisco activity due to accumulation of ions in chloroplast (Soussi *et al.* 1998). In this study, reduction in rate of photosynthesis was associated with a decrease in rubisco activity in all three cultivars. MI-48, however, showed large reduction at both the levels of salinity stress. Chen and Murata (2002) have also reported reductions in rubisco activity under salinity stress. Delfine *et al.* (1998) suggested that salinity stress could reduce the photosynthetic rate either by reducing supply of CO_2 through stomatal closure or by changing mesophyll cell structure.

Plants respond to abiotic stress by decreasing their relative water content (RWC) and osmotic potential (Gadallah 1999). Reduction in the rate of leaf elongation under salt stress is attributed to the changes in leaf water status (Marschner 1995). Salt stress causes reduction in RWC of all the rice cultivars in this study. However, Pokkali and CSR-13 plants were able to maintain higher RWC under salinity compared to MI-48 at both the stages (Table 1). Similarly higher RWC have been reported in drought tolerant cultivars of wheat under stress (Martin *et al.* 1997).

Chlorophyll content is considered as an index to measure leaf injury under salt stress (James *et al.* 2002). We observed no significant changes in chlorophyll content of CSR-13 and Pokkali at S_1 level of salinity but at S_2 level there was marginal reduction in both the genotypes. On the other hand salt sensitive MI-48 showed significant reduction in chlorophyll content at both S_1 and S_2 level of salinity stress (Table 1). Hernandez *et al.* (1995) have reported similar findings in salt sensitive pea cultivars.

Lipid peroxidation is considered as an indicator of the extent of oxidative damage under stress (Bor *et al.* 2003). Lipid peroxidation was measured in terms of TBARS content in the leaves. All the three cultivars showed higher TBARS content in salt treated plants compared to control. However, the increase in TBARS content was highest in

MI-48 followed by CSR-13 and Pokkali (Table 1). Changes in TBARS content have also been reported by Vaidyanathan *et al.* (2003) in salt tolerant and sensitive rice cultivars. Sairam *et al.* (2002) have shown similar changes in TBARS content in both salinity tolerant and sensitive wheat genotypes.

Antioxidant enzymes play significant role in rice plants to protect them against the damaging effect of reactive oxygen species generated during salinity stress (Asada 1992). SOD catalyses, the dismutation of superoxide to H_2O_2, which is detoxified by CAT and/or peroxidases to water and oxygen. Several studies have reported increase in SOD activity in tolerant cultivars compared to susceptible ones under oxidative stress (Hernandez *et al.* 1993). In this study SOD activity increased under salt stress in all the three genotypes and Pokkali showed the highest increase in activity. Salt stress also increased SOD activity in both CSR-13 and MI-48, but the increase in CSR-13 was higher than salt sensitive MI-48 at both the stages (Fig. 1). Singla and Choudhary (1990) have reported similar reduction in SOD activity of sensitive rice cultivars under NaCl stress. On the other hand, Dionisio-Sese and Tobita (1998) have shown reduced SOD activity in salt sensitive rice cultivars with increasing

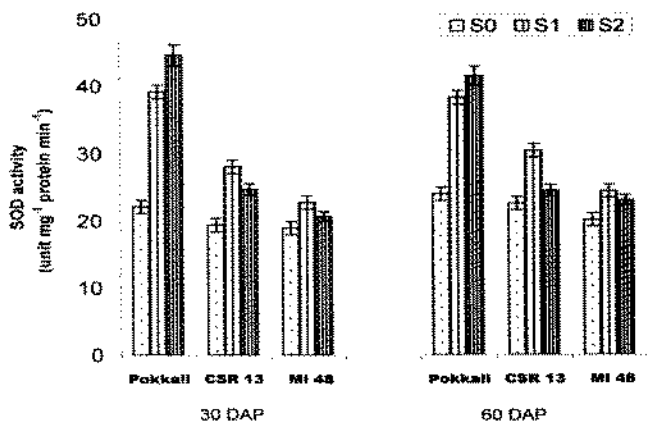

Fig. 1. Changes in superoxide dismutase (SOD) activity in three rice genotypes under salt stress at 30 and 60 days after planting (DAP). S_0= Control, S_1 = 6 dS m^{-1} and S_2 = 12 dS m^{-1}.

magnitude of salt stress and no changes in salt tolerant cultivars. CAT activity showed an opposite trend with regards to salinity stress. Unlike SOD, catalase activity increased in both CSR-13 and MI-48 genotypes with increasing salinity levels but decreased in Pokkali at higher level of salt stress at both stages (Fig. 2). Such

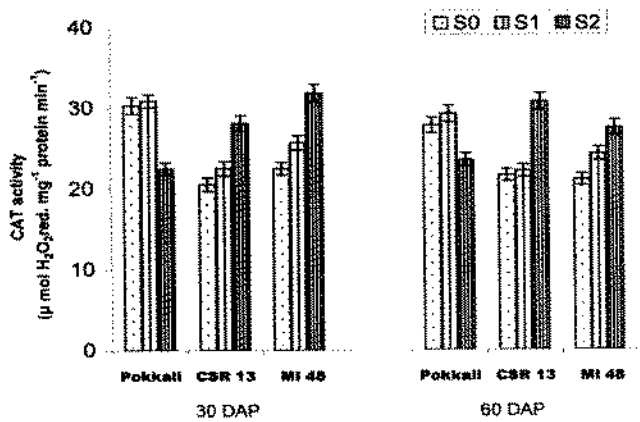

Fig. 2. Changes in catalase activity in three rice genotypes under salt stress at 30 and 60 days after planting (DAP). S_0= Control, S_1 = 6 dS m^{-1} and S_2 = 12 dS m^{-1}.

reduction in CAT activity in Pokkali under salt stress could result in H_2O_2 accumulation and may be associated with its tolerant mechanism through signal transduction. Shim *et al.* (2003) reported that increase in H_2O_2 in plant cell under stress may induce the activity of other enzymes to overcome stress effect. However, some reports show increase in CAT activity in rice under salt stress (Lin and Kao 2000). The changes in CAT may vary according to the intensity of stress, time of assay after the stress and induction of new isozyme(s) (Shim *et al.* 2003). APX activity also increased in both the tolerant and sensitive rice cultivars. However, the increase in APX activity was more in CSR-13 and Pokkali compared to MI-48 (Fig. 3). Increase in APX activity has also been shown in rice under salt stress by others (Vaidyanathan *et al.*

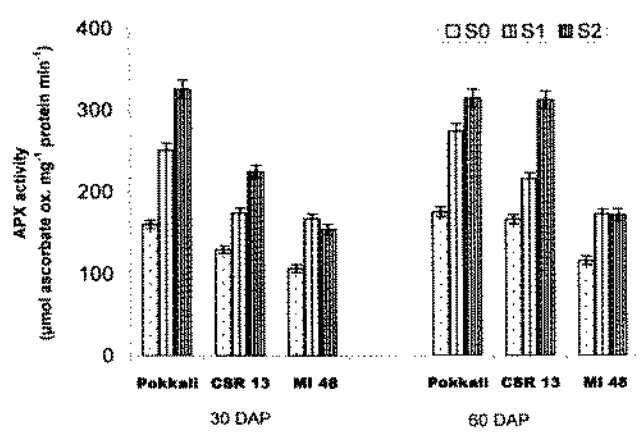

Fig. 3. Changes in ascorbate peroxidase (APX) activity in three rice genotypes under salt stress at 30 and 60 days after planting (DAP). S_0= Control, S_1 = 6 dS m^{-1} and S_2 = 12 dS m^{-1}.

2003). In the present study salt tolerant genotype showed less decrease in P_N and greater increase in SOD and APX activities under salt stress and therefore, these may be considered as important salt tolerant characteristics.

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