SHORT COMMUNICATION

EFFECT OF NaCl STRESS ON PHOTOSYNTHESIS CHARACTERISTICS AND ANTIOXIDANT ENZYMES IN RICE CULTIVARS

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Received on 18th March, 2010, Revised on 26th July, 2010

The effect of NaCl stress on physiological and biochemical characteristics of salinity tolerant CSR 27 and sensitive Pusa Basmati 1 rice cultivar was studied in comparison with CSR10. Thirty-five days old seedling was transplanted in earthen pots with three level of salinity stress (0, 6.0 and 12.0 dSm^{-1}) treatment. Observations were recorded for the rate of photosynthesis (P_N), chlorophyll content, relative water content (RWC) and antioxidant enzymes viz. Superoxide dismutase (SOD) and Catalase (CAT) were determined at 35 and 65 days after transplanting. Rate of photosynthesis and chlorophyll content decreased marginally by NaCl stress in CSR 27 and CSR10 but Basmati 1 showed greater reduction. CSR 10 and CSR 27 maintained higher RWC under NaCl stress compared to sensitive Pusa Basmati 1. SOD activity was highly induced under NaCl stress in CSR10 but CAT activity decreased. In CSR 27, induction of SOD and CAT activities was higher than that of Pusa Basmati 1. The results indicate that maintenance of higher RWC and induction SOD activity under NaCl stress in CSR 27 contributed to its salt tolerant characteristics.

Key words: **Antioxidant enzymes, photosynthesis, relative water content, rice, salinity**

Soil salinity is major constraint limiting agricultural productivity in nearly 20% of cultivated area and half of the irrigated area word 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). Reactive oxygen species (ROS) are regarded as main source of damage to cell of biotic and abiotic stress such as superoxide anion (O^-_2), hydrogen peroxide (H_2O_2) and the hydroxyl radicals (OH^-) particularly synthesized in the chloroplast and mitochondria (Mittler 2002). Plants process a number of antioxidant enzyme like superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) to protect against the damaging effect of ROS (Asada 1992 and Pal *et al*. 2004). Several factors associated with salinity stress can lead to an increase in reactive oxygen species (Apel and Hirt, 2004). Free radical scavenging systems such as superoxide dismutase (SOD; EC 1.15.1.1) can be a critical component of salinity tolerance (Mittova *et al.* 2004) because of their protection of chloroplast function under high salinity (Orcutt and Nisen 2000). Salinity causes a significant decrease in SOD activity in rice seedlings with noticeable differences between varieties (Dionisio-Sese and Tobia 1998).The present investigation therefore, attempts to analyze the physiological and biochemical basis of salt tolerance in rice cultivars differing in sensitive to NaCl stress. Such understanding will be useful in future breeding programmes for developing salt tolerant rice cultivars.

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Thirty five day old seedling of three rice cultivars, viz., CSR 27 (salt tolerant), Pusa Basmati 1 (Salt sensitive) and CSR10 (Salinity tolerant check) were transplanted in earthen pots (35×40cm) lined with double layer of polythene sheet filled with sandy loam soil. Before transplanting pots were irrigated with water (Control), 6.0 and 12.0 dSm^{-1} NaCl solutions to give two salinity stress treatments. The salinity levels were determined in soil samples collected at 10 days interval and mean values expressed as dSm^{-1} were 0.85 (S_0), 6.19 (S_1) and 11.99(S_2). Observations for the rate of photosynthesis (P_N), chlorophyll content, relative water content and antioxidant enzymes were determined at 35 and 65 days after transplanting (DATP). The rate of photosynthesis (P_N) was measured in the top most fully expended leaves at 11am with the help of portable photosynthesis system (Li 6200, LI-COR Inc. Nebraska, (U.S.A). Chlorophyll contents were determined spectrophotometrically following Arnon (1949).

Leaf relative water content (RWC) was estimated following the method of Turner (1981). Leaf samples (0.2 g) were saturated in 100 ml of water for 4 hrs and the turgid weight of leaf samples was recorded. Afterwards the samples were oven dried at 70^0c for one week to measure the dry wt.

SOD activity was assayed on the basis of photochemical reduction in absorbance of nitro-blue tetrazolium (NBT) by enzyme following method of Dhindsa *et al*. (1981). 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 extracts. The experiment was conducted under C.R.D and the data was statistically analyzed following AGRES statistical software version 3.01.

In the present study a decreasing trend in P_N in all the three rice cultivars was observed under salinity stress, exhibited maximum reduction at both the levels of stress (Table 1). The reduction in P_N in CSR10 was not significant at both the levels of stress, whereas in, CSR 27 the reduction in P_N was significant only at S_2 stress level. Salinity reduced the photosynthetic rate either by reducing supply of CO_2 through stomata closure or by changing mesophyll cell structure (Delfine *et al*.1998). 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 at both $S_1$and S_2 level of salinity stress (Table 1). Pal *et al*. (2004) have reported similar finding in salt sensitive rice cultivars. 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, CSR10 and CSR 27 plants were able to maintain higher RWC under salinity compared to Pusa Basmati 1 at both the stages (Table 1). Similarly higher RWC have been reported in drought tolerance of wheat under stress (Maritin *et al*. 1997).

Antioxidant enzymes play a significant role in rice plants to protect them against the damaging effect to reactive oxygen species (ROS) 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 sensitive ones under oxidative stress (Pal *et al*. 2004, Mittova *et al*. 2004 and Khosravinejad *et al.* 2008). In this study SOD activity increased under NaCl stress in all the three cultivars and CSR10 showed the maximum increase in activity. NaCl stress also increased SOD activity in both CSR 27 and Pusa Basmati 1, but the increase in CSR 27 was higher than the salt sensitive Pusa Basmati 1 at both stages (Fig 1). Pal *et al.* (2004) have reported similar reduction in SOD activity in sensitive rice cultivars under salinity stress. Abdel Latef (2010) has reported similar reduction in SOD activity in sensitive wheat cultivars under salinity. On the other hand, Dionisio-Sese and Tobita (1998) have shown reduced SOD activity in salt sensitive rice cultivars with increasing magnitude of salt stress and no changes in salt tolerant cultivars. CAT activity showed an opposite trend with regards to NaCl stress. Unlike SOD, catalase activity increased in both CSR 27 and Pusa Basmati 1 cultivars with increasing NaCl stress levels but decreased CSR10 at higher level of NaCl stress at both stages (Fig.2). Such reduction in CAT activity in CSR10 under NaCl stress could result in H_2O_2

Table 1. Effect of soil salinity on total chlorophyll, rate of photosynthesis and relative water content [RWC (%)] in rice cultivars CSR 10, CSR 27 and Pusa basmati 1 at 35 and 60 DATP in control 6.0 dSm^{-1} and 12 dSm^{-1} salinity level.

Cultivars	Rate of photosynthesis ($\mu mol\ CO_2\ m^{-2}s^{-1}$)		R W C (%)		Total chlorophyll content ($mg\ g^{-1}\ fw$)	
	35 DATP	60 DATP	35 DATP	60 DATP	35 DATP	60 DATP
Control						
CSR 10	14.4	15.3	75.9	80.5	2.51	2.14
CSR 27	16.8	16.2	79.1	81.0	2.82	2.47
Pusa Basmati 1	17.0	16.6	74.0	75.3	2.27	2.55
6 dSm^{-1}						
CSR 10	13.7	14.1	77.4	76.4	2.58	2.16
CSR 27	15.0	14.9	74.1	76.0	2.62	2.62
Pusa Basmati 1	13.3	12.8	68.2	63.7	2.14	2.32
12 dSm^{-1}						
CSR 10	12.7	13.0	71.5	73.4	2.22	2.05
CSR 27	12.4	12.9	71.5	70.5	2.33	2.22
Pusa Basmati 1	10.8	11.0	62.0	64.0	1.94	2.16
S±E	1.21	1.78	0.67	0.63	0.21	0.22
CD (P = 0.05)						
V	3.57	3.51	1.99	1.89	0.62	0.63
S	3.57	3.51	1.99	1.89	0.62	063
V×S	6.18	6.09	3.45	3.55	1.07	1.09

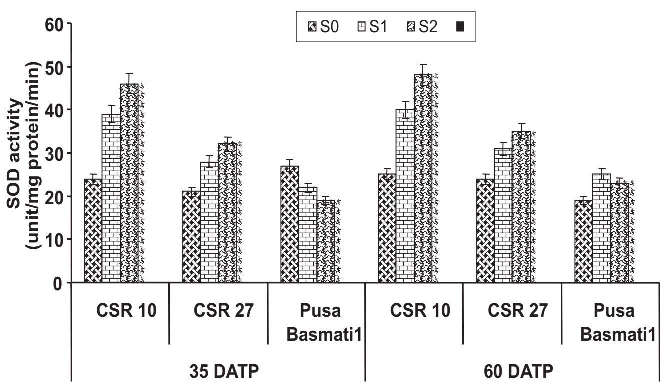

Fig. 1. Effect of soil salinity on the activity of superoxide dismutase in rice cultivars CSR 10, CSR 27 and Pusa basmati 1 at 35 and 60 DATP in control S0 = Control, S_1 = 6 dSm^{-1} and S_2 = 12 dSm^{-1} salinity. Vertical bars show ± SE of mean.

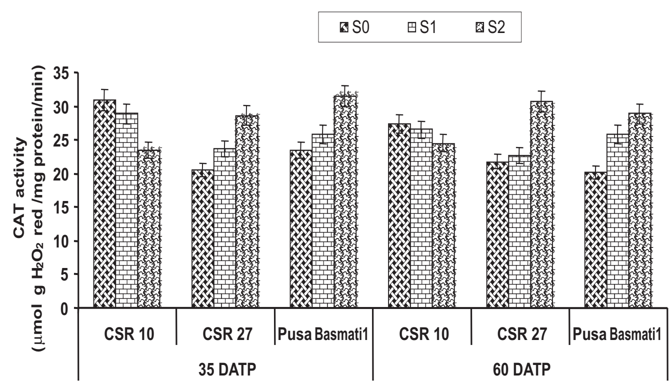

Fig. 2. Effect of soil salinity on the activity catalase in rice cultivars CSR 10, CSR 27 and Pusa basmati 1 at 35 and 60 DATP in control S0 = Control, S_1 = 6 dSm^{-1} and S_2 = 12 d Sm^{-1} salinity. Vertical bars show ± SE of mean.

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, Pal *et al*. 2004 and Abdel Latef 2010). The changes in CAT may vary according to intensity of stress, time of assay after the stress and induction of new isoezyme (s) (Shim *et al*. 2003). In the present study salt tolerant cultivars showed less decrease in P_N and greater in SOD activity under NaCl stress and therefore, these may be considered as important salt tolerant characteristics.

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