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AMELIORATIVE MECHANISMS OF SODIUM CHLORIDE STRESS IN *SESBANIA SESBAN* (L.) MERR.

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SUMMARY

Salinity leads to primary and secondary stresses which includes membrane damage, metabolic disturbance and osmotic stress. Physiological and biochemical responses are induced in plants to ameliorate such stresses. The objective of this study was to obtain a better understanding on salt-tolerance mechanism of *Sesbania sesban* to NaCl. Rate of germination was not affected by the NaCl treatment with 50, 100, 150 and 200 mM. Fresh and dry weights were decreased with increasing NaCl concentration. Greater reduction of chlorophyll b content than chlorophyll a suggested that chlorophyll b is more susceptible to salt stress than chlorophyll a. Accumulation of Na^+ and Cl^- in the old leaves appears to be part of the mechanism to alleviate the salt toxicity. The higher accumulation of proline with increasing NaCl was found in the roots and shoots. *Sesbania* was found to have a greater tolerance to NaCl that could resist NaCl up to 200 mM is probably related with its ability to restrict Na^+ and Cl^- content in roots and translocate higher amount of the ions to shoots. The young and old leaves had a higher constitutive level of SOD, APX, CAT and GR activities with increasing NaCl concentrations. These findings indicated that *S. sesban* has higher levels of antioxidative enzymes both constitutive and induced resulting in greater resistance to oxidative damage caused by NaCl stress.

Key words: **Antioxidants, chlorophyll, salinity stress, *Sesbania sesban*.**

INTRODUCTION

Saline soil is a serious problem which affects the yield of agricultural crops by decreasing both the growth and photosynthesis. It was found that in general, the production of dry matter is severely reduced with salinity in glycophytes, but is still maintained in halophytes. Halophytic plants respond to salinity by accumulating Na^+ and Cl^-, particularly in the older leaves (Apse *et al*.1999). Moreover, sequestering of excess Na^+ and Cl^- ions into the vacuole (Pardossi *et al*.1999) and inducing accumulation of compatible solutes such as proline, glycinebetaine in the cytoplasm are well known mechanism of salt tolerance in the cells (Girija *et al.* 2002). The total uptake of K^+, Ca^{++} and Mg^{++} decreased in salt-sensitive species with increasing salinity. The production of reactive oxygen species (ROS) is one of the biochemical changes during salt stress (Vaidyanathan *et al*. 2003). When plants are subjected to the stress, the balance between the production of ROS and the quenching activity of the antioxidant is upset, often resulting in oxidative stress. Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to this oxidative damage (Bor *et al*. 2003). *Sesbania sesban* is a fast growing shrub with pinnate leaves belongs to Fabaceae. Because of its shallow root system, it can compete with other crops when interplanted. Young leaves are used

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as fodder. The present study was aimed to evaluate the biochemical and physiological basis of tolerance of *Sesbani*a *sesban* to NaCl stress.

MATERIALS AND METHODS

Seeds of *S. sesban* (L.) Merr. were soaked in concentrated H_2SO_4 for 30 min followed by NaClO (1.2% active chlorine) under vacuum for 10 min and washed with distilled water overnight. Fifteen seeds were treated with 10 ml of 0, 50, 100 and 150 mM NaCl solution. Germination rate was determined 7 days after treatment. Germinated seeds were planted in a pot containing 2 kg soil. After the 3rd to 4th leaf stage salt treatment was repeated by adding 50, 100 and 150 mM NaCl solution 50 ml per pot at two-day intervals. All experiments were designed with three replications. The 14 day after strating NaCl treatment seedlings were harvested and separated into roots and shoots for determining the fresh and dry mass. Leaves were excised from the seedlings for the measurement of chlorophyll content according to Chappelle *et al*. (1992). The seedlings were harvested and separated into roots and shoots to estimate total free amino acid and proline as per the method of Moore and Stein (1948) and Bates *et al*.(1973), respectively. Ion contents were measured according to the method described by Kim *et al.* (1999). Antioxidant enzyme activities were determined twelve days after starting NaCl treatment. The leaves were excised and divided into young (4th to 6th) and old (1st to 3rd) leaves for assay as per the standard protocols of ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR) (Nakano and Asada 1981, Havir and Mchale 1987, Beyer and Fridovich 1987, Halliwell and Foyer 1978).

RESULTS AND DISCUSSION

Rate of germination was normal in all the treatments (Table1). Moreover, the root became shorter and hypocotyls turned yellow. Higher NaCl concentration (150 mM) reduced fresh and dry mass of shoots and roots. No leaf injury was apparent even at fairly higher salt concentrations suggesting the salt tolerant nature. The total chlorophyll content was decrease with increasing salt concentration (Table 2). The decrease in chl b content however, was more than chl a. This resulted in the increase in chlorophyll a/b ratio at higher salt concentration, indicating that Chl b is more sensitive to salinity than Chl a. Similar observations were made by Ma *et al*. (1997). NaCl stress decreased total chlorophyll content possibly by increasing the activity of Chl degrading enzyme cholorophyllase, inducing the destruction of chloroplast and instability of pigment-protein complexes (Singh and Dubey 1995). Thus the results indicate that chl b is a good indicator of salt stress.

Table 1. Effect of different concentrations of NaCl on germination and fresh and dry weights of seedlings (g/seedling) of *Sesbania sesban* 14 days after starting NaCl treatment

		NaCl Conc.			
Parameter		**0 mM**	**50 mM**	**100 mM**	**150 mM**
Germ. (%)		100±1.1	98±0.9	94±1.3	93±1.3
Root	Fresh weight	4.2±0.6	4.4±0.5	2.5±0.4	2.2±0.2
	Dry weight	0.4±0.05	0.4±0.03	0.3±0.02	0.3±0.02
Shoot	Fresh weight	3.7±0.6	4.1±0.5	2.7±0.4	2.5±0.2
	Dry weight	0.7±0.1	0.7±0.1	0.4±0.1	0.4±0.1

Table 2. Effect of salinity stress on chlorophyll content μg/g fw) in the leaves of *Sesbania sesban*

NaCl	Chl a	Chl b	Chl a+b	Chl a/b
0 mM	144.5± 1.6	221.1± 3.1	365.6± 2.6	0.6± 0.02
50 mM	137.7± 1.0	134.5± 12.9	272.2± 12.9	1.0± 0.12
100 mM	128.6 ± 5.4	38.1± 2.9	166.7± 7.2	3.3±0.05
150 mM	115.4± 3.0	35.2± 1.5	160.6± 4.4	3.2± 0.06

Na^+ and Cl^- distribution increased with increasing salinity. However, the accumulation was noted more in shoots than in roots (Table 3). This is a physiological adaptation to reduce the salt toxicity in and away from the root cells so that the plant could survive even at the higher salinity levels. More precise determinations of the distribution of the ions in shoot parts showed that Na^+ and Cl^- ions accumulated more, particularly in the old leaves (Fig. 1a and b). This function is considered to be effective in avoiding huge accumulations of the toxic salts

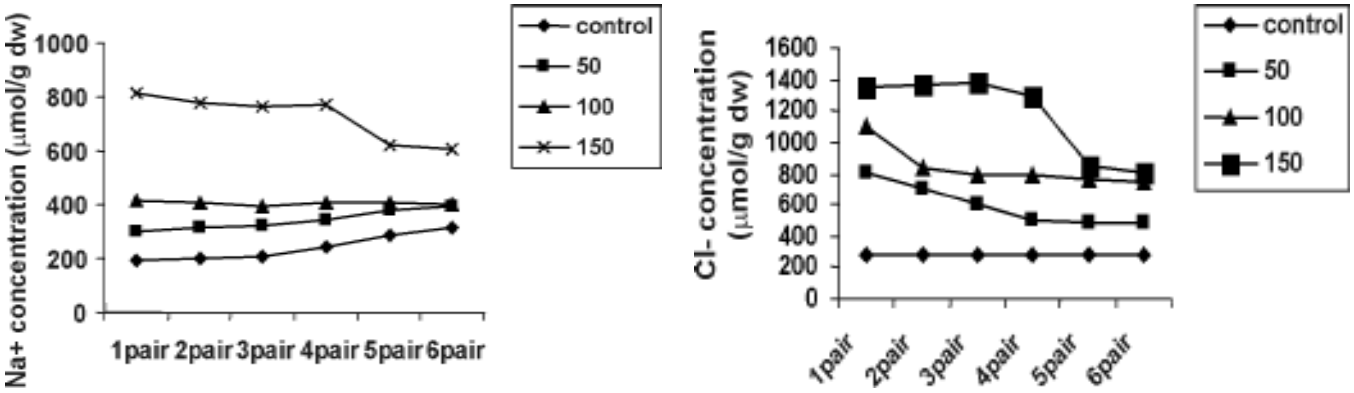

Fig. 1. Distribution of Na^+ (a) and Cl^- (b) in 1st to 6th pair of leaves in *S. sesban* 14 days after starting NaCl treatment

in the growing young leaves (Wahome *et al*. 2001). NaCl did not affect the Ca^{++} and Mg^{++} uptake in roots and shoots but potassium content showed instability whereas phosphorus tended to increase with increase in salt concentrations (Table 3). Total free amino acids increased with salinity in both shoot and root. At the highest concentration, although the total amino acids and proline were clearly built up in both the shoots and roots, their accumulation was more obvious in shoots. The proline content was less at low concentrations but showed a significant increase at higher concentrations (Fig. 2). Higher proline accumulation in the species may contribute to the alleviation of NaCl stress (Girija *et al*. 2002).

Table 3. Ion contents (µmol/g dw) in *S. sesban* 14 days after starting NaCl treatment. The data are the means of 3 replicates ± S.E.

Ion	NaCl (mM)			
	0	50	100	150
		Root		
Na+	310±22.4	434±19.1	578±16.2	645±33.8
K+	456±9.2	223±10.4	198±6.9	168±5.4
Mg++	123±9.4	132±1.1	139±3.4	144±2.4
Ca++	44±9.7	34±2.4	33±5.4	32.5±4.4
P	334±19.4	365±12.2	389±11.3	396±20.4
Cl-	336±11.4	354±10.1	454±21.0	501±9.3
		Shoot		
Na+	411±3.4	1540±18.8	1984±66.4	2404±231.4
K+	987±320.1	404±29.4	399±19.4	378±2.4
Mg++	199±12.4	178±20.4	176±23.6	163±2.9
Ca++	169±33.4	176±15.4	199±9.4	204±4.4
P	389±12.4	523±12.4	634±22.4	765±9.4
Cl-	354±10.1	1054±20.1	1354±7.8	2024±20.8

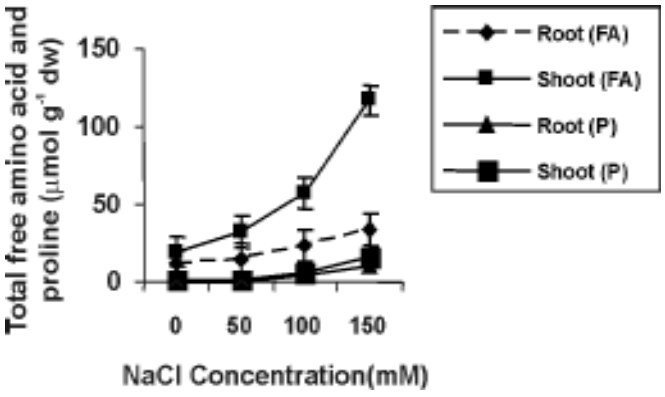

Fig. 2. Total free amino acid (FA) and proline (P) content in *S. sesban* 14 days after starting NaCl treatment

Antioxidant enzyme (SOD, APX and CAT) activities in the young and old leaves determined 12 days after treatment were induced with increasing salt concentrations, whereas, the GR activity did not change (Table 4). The oxidative stress is considered to be due to an increased production of ROS. In non-treated control, the greater activity of antioxidative enzymes was found in younger leaves than in older leaves. Hence, constitutive and /or induced antioxidative enzymes such as SOD, APX, CAT and GR are needed to protect plant

Table 4. Effect of salinity stress on antioxidant enzyme activities: Ascorbate peroxidase (APX, µmol decomposed/ g fw/min), catalase (CAT, µmol H_2O_2 decomposed/g fw/min), superoxide dismutase (SOD, unit/g fw), glutathione reductase (GR, µmol NADPH oxidised/g fw/min) in young (4^{th} to 6^{th}) and old (1^{st} to 3^{rd}) leaves of *S. sesban* 12 days after strating NaCl treatment

Antioxidant	NaCl(mM)					
enzymes	0	50	100	0	50	100
		Young leaf			Old leaf	
APX	5.8± 0.5	7.5± 0.8	8.8± 0.5	2.9± 0.4	3.8± 0.5	4.7± 0.3
CAT	978.6±34.1	1065± 44.2	1123± 53.5	521± 145	1012± 103	1127± 35
SOD	892± 109	906± 12.9	1323± 156	478± 15.5	598± 50.3	706± 46
GR	1± 0.1	0.8± 0.1	0.78± 0.0	1.1± 0.1	1.0± 0.0	1.1± 0.0

tissue from the oxidative damage. The data reveals that *S. sesban* has a better protection system against oxidative damage caused by salinity stress. Several previous studies also reported that salt-tolerant cultivars of tomato and beet root had higher constitutive levels of antioxidant enzymes (Bor *et al*. 2003). High constitutive and induced levels of SOD and CAT in the leaves indicate that their combined action is an effective scavenging mechanism to abate the toxic effect of O_2^- and H_2O_2 in the leaf cells. In plant cells, the most important substrate for H_2O_2 detoxification is reduced ascorbate. APX uses reduced ascorbate as the electron donor for the reduction of H_2O_2 and also acts together with the strong catalysts, monodehydroascorbate (MDHAR), dehydroascorbate (DHAR) and GR (Noctor and Foyer 1998). The removal of H_2O_2 through the reaction is known as the ascorbate-glutathione cycle (Noctor and Foyer 1998). Higher activity of APX in the leaves also seemed to be responsible for scavenging of H_2O_2. Because GR activity was not changed, the involvement of the reaction to detoxify H_2O_2 through the ascorbate-glutathione cycle was not clear. A better understanding of antioxidant defense system is therefore, required.

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