Plant Archives Vol. 15 No. 2, 2015 pp. 817-822 *ISSN 0972-5210*

DIFFERENTIAL RESPONSES OF SUGARCANE (*SACCHARUM OFFICINARUM* L.) VARIETIES EXPOSED TO SALINITY UNDER A HYDROPONIC SYSTEM

R. Anitha*, P. Christy Nirmala Mary, M. Antony Joseph Ravi Savery, N. Sritharan and R. S. Purushothaman

Sugarcane Research Station, Tamil Nadu Agricultural University, Cuddalore (Tamil Nadu), India.

Abstract

A hydroponical experiment was conducted to assess salt stress responses of sugarcane (*Saccharum officinarum* L.) var. CoC 671 and CoC 24 to evaluate the possible interaction of various level of salinity (0,150, 200 mM NaCl) and its impact on growth parameters *viz.*, shoot length, root length, root volume, leaf area and also the physiological parameters *viz*., chlorophyll content, cell membrane stability, relative water content, nitrate reductase activity, proline content and catalase. With increase in NaCl concentration of the shoot, root length and leaf area decreased in both varieties CoC 671 and C0C 24 by 41%, 35.88%; 28.93%, 42.36% and 65.6%, 51.8% respectively, when compared to control. Total chlorophyll, cell membrane stability, relative water content and nitrate reductase activity also decreased drastic all under NaCl stress than control. The antioxidant enzyme catalase and the amino acid proline content were found to be increase in CoC 671 and CoC 24 than control. In this study, it was observed that CoC 24 displayed higher tolerance to NaCl than CoC 671.

Key words : Sugarcane, NaCl, CoC 24 and CoC 671.

Introduction

Salinization is one of the most devastating forms of land degradation threatening food production worldwide, especially in arid and semi-arid countries. However, climate change predictions indicated less rainfall and higher temperatures in the future in most of the agricultural regions. So, experts worry that the changes will lead to even more saline lands and predict that salinity will increase from 4 to 9 dSm^{-1} in the future. Progress in developing salt tolerant varieties has been very slow because of less knowledge on the mechanism of salt damage and complex nature of salt tolerance. Sugarcane is a typical glycophyte exhibiting stunted growth or no growth under salinity, with its yield falling to 50%or even more of its true potential (Subbarao and Shaw, 1985), which could possibly be due to the accumulation of toxic ions. Thus, understanding the adaptive mechanisms of each crop becomes necessary to improve or produce the salt resistant genotypes. Salinity may cause damage to the plants through osmotic stress, nutrient imbalance and specific ion toxicity (Munns *et al*., 1986). Assessment of the available germplasm against salt stress should become valuable resource for its successful cultivation in problem soils. Salinity in the root zone of sugarcane decreases sucrose yield, through its effect on both biomass and juice quality (Lingle and Wiegand, 1996). Due to this, losses occur in growth rate and sugar content of the plant (Rozeff, 1995).

Plant responses to salt stress are complex involving many genetic networks and metabolic processes and these depend on the inherent salt tolerance of the plant, concentration of salt and the duration of exposure (Hasegawa *et al*., 2000; Munns and Tester, 2008). Plant adaptations to salinity are of three distinct types: osmotic stress tolerance; Na^+ exclusion; and tissue tolerance, that is, tolerance of tissue to accumulated Na^+, and possibly Cl^- (Munns and Tester, 2008). Additionally, osmolytes (betaines and proline) and antioxidant systems (peroxidases like ascorbate peroxidase, guaiacol peroxidase, catalase, and superoxide dismutase) are also important (Greenway and Munns, 1980; Hasegawa *et al*., 2000; Ashraf and Harris, 2004; Patade and Suprasanna, 2009). In crop improvement programs, it is often desirable to have a good and reliable method for screening large plant population to isolate salt tolerant

***Author for correspondence:** E-mail: anithasrs2014@gmail.com

clone or mutant. In this light of view, investigation was done by using hydroponic method, sugarcane cultivars (CoC 671 and CoC 24) were examined to study their differential physiological changes and growth parameters.

Materials and Methods

A hydroponic culture with the use of half strength Hoagland's solution (Hoagland and Arnon, 1950) was conducted by using three treatments (NaCl), which include 0, 150, 200mM NaCl while plants kept in ½ strength Hoagland's solution without NaCl supplementation served as the control, for the 30 days old plant. The experiment was carried out at Sugarcane Research Station, Cuddalore, during the year 2015 using a promising sugarcane varieties *viz*., CoC 671 and CoC 24 with five replication by adapting factorial randomized block design. The observations on physiological parameters and biochemical analyses were recorded in three plants for each treatment on 50th days.The data were subjected to analysis of variance (ANOVA), to test the significance of the parameters.

The electrical conductivity (EC) and pH of the soil, in relation to the application of different treatments was also measured before and after the completion of the experiment. For this purpose, a soil sample (10g) from cup of each treatment was taken randomly. Sand was mixed in a small amount of water and allowed to set for 5 min. Then the water was separated and readings for EC and pH were recorded using EC and pH meters, respectively. The shoot, root length, root volume and leaf area were recorded by using ruler with units as cm and root volume were measured by using one litre measuring cylinder with units as ml per plant and leaf area per plant was measured by K constant value (0.6247) along with length and breadth of leaf after 50 days. Chlolrophyll was extracted in 80% acetone from the leaf samples according to the method of Arnon (1949). Extracts were filtrated and content of total chlorophyll was determined by spectrophotometry at 652nm and it was expressed as mg/g of fresh weight.

For estimating, Relative water content (RWC), leaf bits were taken and fresh weight (FW, gm) was recorded before floating the bits in distilled water for 5 hrs for attaining full turgidity. After recording turgid weight (TW, gm) the samples were dried in hot air oven to a constant weight and dry weight (DW, gm) was recorded. The RWC was estimated using the formula

$$\text{RWC (\%)} = \frac{(\text{FW} - \text{DW})}{(\text{TW} - \text{DW})} \times 100$$

Membrane stability was measured as described by Lutts *et al*. (2004) with a few modifications. Plant material (0.3 g) was washed with distilled water, placed in tubes with 15 ml of deionised water and incubated for 2 h at 25°C. Subsequently, electrical conductivity of the solution (L_1) was determined. Samples were then autoclaved at 120°C for 20 min and the final conductivity (L_2) was measured after equilibration at 25°C. Membrane stability were calculated by following formula: Membrane stability (%) = $(L_1/L_2) \times 100$. Nitrate reductase activity was assayed by following method of Nicholas *et al*. (1976).

Proline is an important compatible osmolyte and osmoprotective compound, acting as molecular chaperone in osmotic adjustment during osmotic stress. Determination of proline content by acid ninhydrin is simple, reliable and quantitative, does not need sophisticated instrumentation or expensive reagents and has been tested in plants (Bates *et al.*, 1973).

At acidic pH ninhydrin can form a red product with proline and ornithine, which can be used for the estimation of the concentration of these amino acids in pure solution.

Protocol : Fresh leaf materials (0.1g) were homogenized with 5 ml sulfosalicylic acid (3.0%) w/v with a mortar and pestle. Samples were centrifuged at 3000 rpm for 10 min. Supernatant was adjusted to 5ml with sulfosalicyclic acid, 5ml glacial acetic acid and 5ml acidic ninhydrin (0.1% in acetone) were added. Reaction mixture was shaken and heated in water bath for 30min. Mixture was cooled and then extracted with 10ml toluene in separating funnel. Absorbance of the toluene layer was recorded at 520 nm. A calibration series of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg ml^{-1} of proline was also run and a standard curve was plotted and the concentration of the unknown sample was calculated for the proline content with reference to the standard curve. The CAT (catalase) activity (EC:1.11.1.16) was determined by employing the method suggested by (Gopalachari, 1963) estimating the residual H_2O_2 by oxidation with $KMnO_4$ titrimetrically and expressed as mg H_2O_2 g^{-1}.

Result and Discussion

Salinity stress significantly decreased the growth of sugarcane. Root and shoot length decreased with increasing levels of salinity. In the variety CoC 671 the shoot length was found to decrease by 41% as compared with the CoC 24 (35.88%). Incidentally, it was noticed most significant decline in root length at 200mM of NaCl in CoC 671 as compared to CoC 24 (table 1). Massai *et al*. (2004) have found that salinity post pones plant growth under reduction of photosynthesis effects, and causes closing of stomata and reduction of water entrance into the plant so that it causes duplicate reduction in plant weight. Younis *et al*. (2010) reported that the growth

Table 1 : Growth parameters of sugarcane genotypes under salt stress.

Treatment	Genotypes	Shoot length (cm)	Root length (cm)	Leaf Area (cm^2)	Root volume (ml)
Control	CoC 671	94.25	31.8	67	45
	CoC 24	143.5	28.8	75	85
	Mean	**118.875**	**30.3**	**40.5**	**65**
150mM NaCl	CoC 671	58.4	28.3	39.1	24
	CoC 24	94	24.9	42.5	60
	Mean	**76.2**	**26.6**	**40.8**	**42**
200mM NaCl	CoC 671	55.6	22.6	23	32
	CoC 24	92	16.6	36.1	62
	Mean	**73.8**	**19.6**	**29.55**	**47**
Stage mean		**89.625**	**25.5**	**36.95**	**51.33**
		CD	**CD**	**CD**	**CD**
Treatments		3.06	1.05	1.25	2.10
Variety		3.59	1.02	1.12	2.04
T × V		7.12	2.15	2.75	4.30

reduction caused by salinity stress is due to inhibited apical growth in plants as well as endogenous hormonal imbalance. The leaf area per plant was significantly reduced under salt stress, while CoC 24 showed 51.8% reduction over control where as CoC 671 exhibited 65.6% reduction. Thus CoC 24 possessed higher leaf area than CoC 671.

A linear reduction in total chlorophyll content by 45% and 20% in CoC 671 and CoC 24 respectively with increasing salinity level (200mM) was observed. The reduction of chlorophyll due to stress is related to the increase of production of free oxygen radicals in the cell. These free radicals cause peroxidation, disintegration and reduction of chlorophyll content in plant under stressful conditions. Sritharan and Malliga vanangamudi (2006) reported that total chlorophyll content was declined in susceptible rice seedlings under salt stress. Salinity stress decreased the membrane stability; however there was no significant difference among the salinity level (table 2). Membrane stability reflects the changes of cell membrane structure under stress. The results of the present study are in agreement with Bayat *et al*. (2012), who determined that membrane stability of *Calendula officinalis* plant was intensively decreased by salt stress treatment. These results suggested cell membrane structure of sugarcane leaves under salinity stress received damage after treatment with NaCl. The results of this study showed that both growth parameters and physiological attributes of sugarcane were adversely affected by salt stress. Nitrate reductase (NRase) activity at 50 days old plant showed drastic reduction in both cultivars over control and among them, CoC 24 expressed higher NRase activity than CoC 671 under salt stress (200 mM). Similar findings were also reported by Sritharan and Malliga Vanangamudi (2006).

A significant rise in the level of proline was observed in both the varieties when the NaCl concentration increase over 200mM, 31.42% hike was observed in CoC 671 and 48.33% increase in CoC 24 (table 3). Among the varieties, the differential accumulation of proline may be due to the response of a variety towards the environment (Hosseini *et al*., 2010). Although, it has been shown by various group that over accumulation of proline is non-specific in nature towards stress (Hosseini *et al*., 2010; Errabii *et al*., 2007 and Ashraf and Hassis, 2004), it could also be due to higher amount of cell injury, thus proving them to be salt susceptible (Errabii *et al*., 2007; Lu *et al*., 2007; Aazami *et al*., 2010). The overproduction of proline may also mean a greater stress impact in CoC 671 as compared to CoC 24, thus, rendering higher salt tolerance in CoC 24 when compared to CoC 671 under 200mM level of NaCl stress. Proline has also been reported to accumulate in order to maintain osmotic potential of the plant cell without affecting other molecules or enzymes enabling tolerance of cells towards salt (Stewart and lee 1973; Greenway and Munns, 1980).

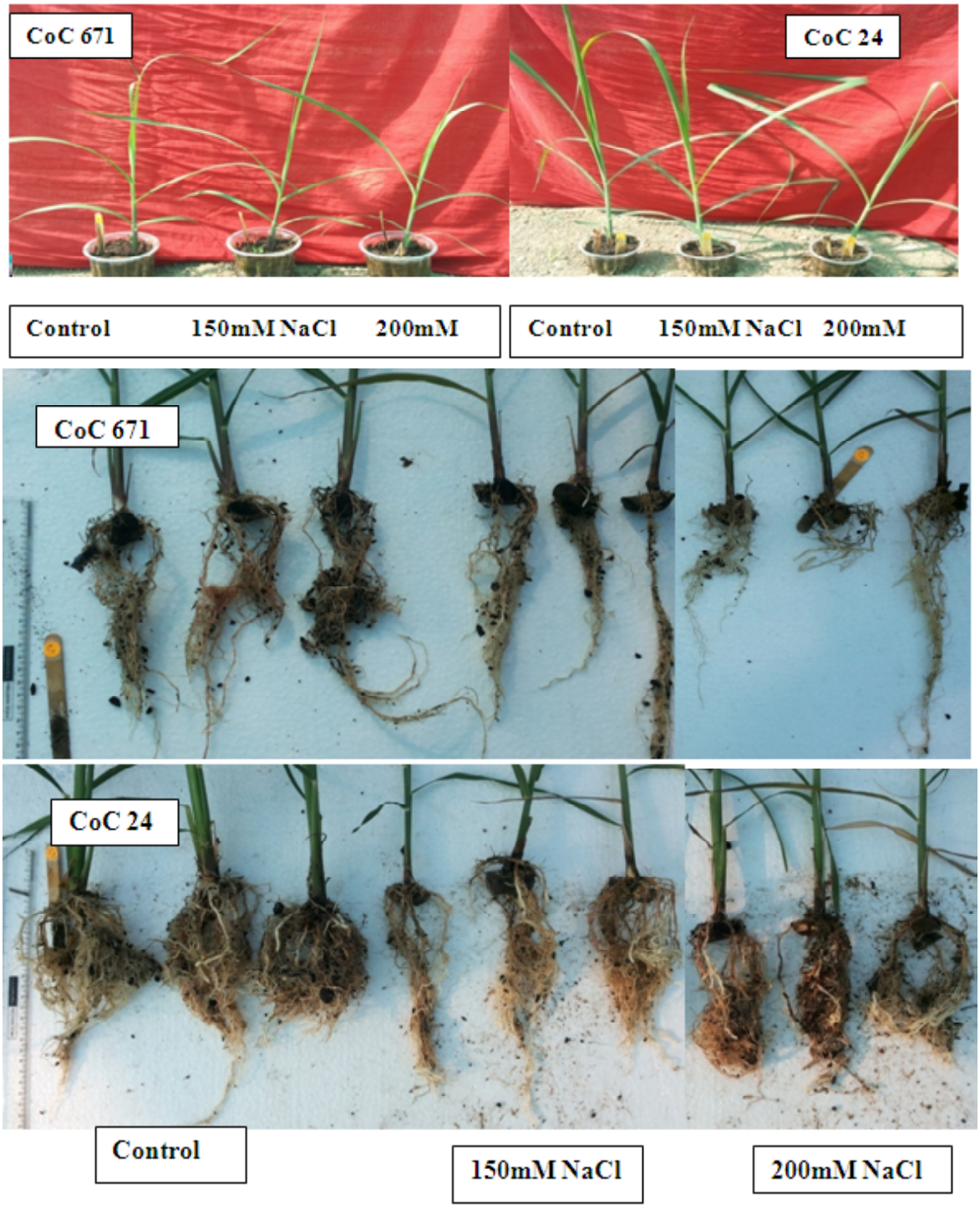

Fig. 1 : Salt-induced shoot and root length in sugarcane CoC 671 and CoC 24 plants. Hydropinically grown sugarcane (50-days-old) seedlings were exposed to salt stress for 14 days.

Additionally, proline also acts as scavenger of free radicals, thus, buffering the redox cell conditions, besides acting as protein hydrotope there by lowering cytoplasmic acidosis, and maintaining required NADP+/NADPH ratios compatible with metabolism (Ashraf and Foolad, 2007). Proline is one of the major compatible solutes, which may help to maintain relatively high water content necessary foe plant growth and cellular function. It has also been reported that proline may act as a buffer protecting the cells from large changes in cytosolic pH, which may accompany cell desiccation (Slocum *et al*., 1984).

Similar to the proline, the activity of the antioxidant enzyme catalase was observed to increase gradually with

Table 2 : Physiological traits of sugarcane genotypes under salt stress.

Treatment	Genotypes	Total chlorophyll (mg/g)	Cell Membrane Stability(%)	Relative water content (%)	Nitrate reductase activity (µg of No_2/g/hr)
Control	CoC 671	0.743	77	20	500
	CoC 24	0.585	79	25	700
	Mean	**0.664**	**78**	**22.5**	**600**
150mM NaCl	CoC 671	0.670	69	13.88	400
	CoC 24	0.480	64	20	600
	Mean	**0.575**	**66.5**	**16.94**	**500**
200mM NaCl	CoC 671	0.407	55.6	12.24	240
	CoC 24	0.466	50.1	18.42	400
	Mean	**0.4365**	**105.6**	**15.33**	**320**
	Stage mean	**0.5585**	**83.366**	**18.256**	**473.3**
		CD	**CD**	**CD**	**CD**
Treatments		0.013	2.78	0.09	13.02
Variety		0.015	2.69	1.01	17.05
T × V		0.020	6.15	1.69	40.15

Table 3 : Biochemical parameters of sugarcane genotypes under salt stress.

Treatments	Genotypes	Proline (µg/g of tissue)	Catalase (µg of H_2O_2/g of tissue)
Control	CoC 671	192	45.05
	CoC 24	248	55.01
	Mean	**220**	**50.03**
150mM NaCl	CoC 671	256	53.21
	CoC 24	364	65.28
	Mean	**310**	**59.245**
200mM NaCl	CoC 671	280	50.33
	CoC 24	480	67.81
	Mean	**380**	**59.07**
	Mean	**303.33**	**56.115**
		CD	**CD**
Treatments		11.25	2.52
Variety		14.65	2.24
T × V		30.08	5.01

respect to increase in NaCl concentration (*i.e.*) 18.9% increase was recorded in CoC 24 than CoC 671 (10.49%) over control. It was observed that tomato under high salt concentration showed higher antioxidant enzyme activities such as SOD, catalase, peroxidase, glutathione reductase and GST (Rodriguez-Rosales *et al*., 1999). The activity of catalase enzyme increases significantly with the increment in NaCl stress (Bor *et al*., 2002), thus regulating the level of other peroxidases necessary for preventing peroxidation of organelle and cell membrane (Lin and Kao, 1999). From the studies, it could be concluded that with respect to the growth, physiological and biochemical parameters CoC 24 exhibited higher tolerance under salt stress than Coc 671 by means of higher membrane stability, more proline content and better catalase activity. Further, the study also suggests that hydroponic culture of plants can be a useful tool to screen the sugarcane plants for salinity stress.

Acknowledgement

Author is thankful to R. S. Purushothaman, Professor and Head, Sugarcane Research Station, Tamil Nadu Agricultural University, Cuddalore, Tamil Nadu, India for his kind guidance, motivation and unconditional support for this work.

References

Aazami, M. A., M. Torabi and E. Jalili (2010). *In vitro* reponse of promising tomato genotypes for tolerance to osmotic stress. *Afr. J. Biotechnol*., **9(26)** : 4014-4017.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts, polphennoloxidase in Beta vulgaris L. *Plant Physiology* **24(1)**, 1-15. http://dx.doi.org/10.1104/pp.24.1.1

Ashraf, M. and M. A. Foolad (2007). Improving plant abiotic-stress resistance by exogenous application of osmoprotectants glycine betaine and proline. *Env. Exp. Bot*., **59** : 206-216.

Ashraf, M. and P. J. C. Harris (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant Sci*. ,**166** : 3-16.

Bates, L.S., R. P. Waldern and I. D. Teare (1973). Rapid determination of free proline for water stress studies. *Plant Soil*, **39** : 205-208.

Bayat, H., M. Alirezaie and H. Neamati (2012). Impact of exogenous salicylic acid on growth and ornamental characteristics of calendula (*Calendula officinalis* L.) under salinity stress. *Journal of Stress Physiology & Biochemistry*, **8(1)** : 258-267.

Bor, M., M. Ozdemir and I. Turkan (2002). The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritime* L. *Plant Sci*., **164** : 77-84.

Errabii, T., C. Bernard, Gandonou, H. Essalmani, J. Abrini, M. Idaomar and N. S. Sehaji (2007). Effects of NaCl and mannitol induced stress on sugarcane (*Saccharum* spp.) callus cultures. *Acta Physiol. Plant*, **29** : 95-102.

Gopalachari, N. C. (1963). Changes in the activities of certain oxidizing enzymes during germination and seedling development of (*Phaseolus mungo*) and (*Sorghum vulgare*). *Indian J. Expl. Biol.,* **1** : 98-100.

Greenway, H and R. Munns (1980). Mechanisms of salt tolerance in nonhalophytes. *Ann. Rey. Plant Physiol.*, **31** : 149-90.

Hasegawa, P. M., R. A. Bressan, Zhu J-K and H. J. Bohnert (2000). Plant cellular and molecular responses to high salinity. *Ann. Rev. Plant Biol*., **51** : 463-99.

Hoagland and Arnon (1950). *The water-culture method for growing plants without soil*. Berkeley, Calif. : University of California, College of Agriculture, Agricultural Experiment Station.

Hosseini, T., F. Shekari and M. Ghorbanli (2010). Effect of salt stress on ion content, proline and antioxidative enzymes of two safflower cultivars (*Carthamus tinctorius* L.). *J. Food Agric. Environ*., **8(2)** : 1080-1086.

Nicholas, J. C, J. E. Harper and R. H. Hageman (1976). Nitrate Reductase Activity in Soybean. I. Effects of Light and Temperature. *Plant Physiology*, **58(6)**, 731-735. doi:10.1104/pp.58.6.731

Lin, C. and C. H. Kao (1999). Effect of NaCl stress on H_2O_2 metabolism in rice leaves. *Plant Growth Regul*., **30** : 151-155.

Lingle, S. E. and C. L. Weigand (1996). Growth and yield responses of sugarcane to saline soil II. Sucrose biochemistry in individual internodes. *Proc. Int Am. Sugarcane Seminars*, pp. 93-102.

Lutts, S., M. Almansouri and J. M. Kinet (2004). Salinity and water stress have contrasting effects on the relationship between growth and cell viability during and after stress exposure in durum wheat callus. *Plant Science*, **167(1)** : 9-18. http://dx.doi.org/10.1016 /j.plantsci.2004.02.014

Masssai, R., D. Remorin and H. Tattini (2004). Gas exchanges, water relation and osmotic adjustment in two scion root stock combination of pronus under water various salinity. *Plant and soil*., **259** : 153-162.

Munns, R. and A. Termaat (1986). Whole-plant responses to salinity. *Aust. J. Plant Physiol*., **13** : 143-160.

Munns, R. and M. Tester (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol*., **59** : 651-81.

Patade, V. Y., P. Suprasanna and V. A. Bapat (2008). Effects of salt stress in relation to osmotic adjustment on sugarcane (*Saccharum officinarum* L.) callus cultures. *Plant Growth Regul*., **55** : 169-173.

Rodriguez-Rosales, M. P., L. Kerbeb, P. Bueno and J. P. Donaire (1999). Changes induced by NaCl in lipid content and composition, lipoxygenase, plasma membrane H+-ATPase and antioxidant enzyme activities of tomato (*Lycopersicon esculentum* Mill) calli. *Plant Sci*., **143** : 143-150.

Rozeff, N. (1995). Sugarcane and salinity – a review paper. *Sugarcane*, **5** : 8-19.

Slocum, R. D., R. Kaur – Sawhney and A. W. Galstan (1984). The physiology and biochemistry of polyamines in plants. *Arch. Biochem and Biophy*., **235** : 283-303.

Sritharan, N. and Mallika Vanangamudi (2006). Screening of rice genotypes for salt tolerance. *Plant Archives*, **6(2)** : 815-818.

Stewart, G. R. and J. A. Lee (1974). The Role of Proline Accumulation in Halophytes. *Planta (Berl.)*, **120** : 279-289.

Subbarao, M. and M. A. E. Shaw (1985). A review of research on sugarcane soils of Jamaica. *Proc. Meeting West Indies Sugar Technol*., **2** : 343-55.

Younis, M. E., M. N. A. Hasaneen and A. M. S. Kazamel (2010). Exogenously applied ascorbic acid ameliorates detrimental effects of NaCl and mannitol stress in *Vicia Faba* seedlings. *Protoplasma*., **239(1)** : 39-48. http://dx.doi.org/10.1007/s00709-009-0080-5