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Effect of Polyamine on Physio-Biochemical Traits in Sugarcane Grown under Sodic Soil

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ABSTRACT

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The present investigation entitled with “Polyamine effect on biochemical events in sugarcane grown on sodic soils” was carried out to study the efficacy of polyamines viz., putrescine and spermidine to ameliorate the effect of soils sodicity by analysing the lipid peroxidation, chlorophyll a content, chlorophyll b content, total chlorophyll content, reducing sugars, non-reducing sugar, and total sugars. The leaves of the two sugarcane cultivars viz., the salt tolerant CoM 0265 and salt susceptible CoC 671 grown on sodic soils and polyamines were applied at 0, 100 and 500 μ M concentration. The two foliar application of these polyamines were carried out 45 days after planting with 8 days interval. The leaf samples were collected after 65 days after planting for further biochemical analysis. The effect of polyamines was comparatively higher in CoM 0265 for lipid peroxidation than the CoC 671. The proline and glycine betaine increased however, sugars were decrease in the sugarcane leaves in both the cultivars when grown in sodic soils. The application of polyamines increased the proline, glycine betaine and sugars content in these two cultivars with increase in concentration of polyamines. Interestingly the proline content was found to be higher in salt susceptible cultivar CoC 671 at 500 μ M polyamine application than the salt tolerant cultivars CoM 0265.

Introduction

Saccharum has been derived from Sanskrit word *Sarkara* meaning sugar. Sugarcane is tropical and subtropical world crop, which is propagated through sets. Sugarcane (*Saccharum officinarum*) is one of the most important commercial crops in the world. India is the second position in area, production

and productivity in the world next to Brazil. Globally in world area during 2016-2017 was - 20.42 million ha, production - 1333 million tons and Productivity - 65.2 tons/ha respectively. In India Area - 4.608 million ha, production - 289.6 million tons and productivity - 62.2 tons/ha. In India total area of cultivation of sugarcane is 4.38 Mha, production is about 306.72 MT and average

yield of sugarcane is 69.88 ton ha⁻¹ during the year 2016-17. In Maharashtra the total area cultivation of sugarcane is 0.63 Mha and which is about 14.42 percent of all India sugarcane cultivation. The production of sugarcane in Maharashtra is 50.64 MT which is about 16.31 percent of all India production. The average yield of sugarcane is 80 ton ha⁻¹ (Anonymous, 2018).

Maintaining crop yields under adverse environmental stresses is probably the major challenge facing modern agriculture. Due to the excessive irrigation the soils in the Maharashtra have become saline. Numerous attempts have been made to improve the salinity tolerance of a variety of crops by traditional breeding programs, but the progress to develop such salt-resistant plants is very slow and commercial success is limited (Duan *et al.*, 2008). Sugarcane is a glycophyte, with its yield falling to 50% or even more of its true potential which could possibly be due to the accumulation of toxic ions. Being highly crossbred, sugarcane exhibits a significant genetic variability in nature (Wahid *et al.*, 1997).

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 be increased 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. 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).

Polyamines (PAs), including the spermidine, spermine and putrescine are now regarded as plant growth regulators and secondary messenger in signaling pathways (Kusano *et al.*, 2008). Because of their cationic nature at physiological pH, PAs are able to interact with proteins, nucleic acids, membrane phospholipids and cell wall constituents, thereby stabilizing these molecules. PAs have been reported to be involved in defense response to biotic and abiotic stresses (Alcázar *et al.*, 2010). Exogenously applied PAs have been reported to substantially enhance salt tolerance in rice plants (Chattopadhyay *et al.*, 2002).

During the past few years, a number of genes encoding PA biosynthetic enzymes have been isolated in different plants. Numerous transgenic plants with perturbed PA metabolism have been produced to elucidate their cellular functions furthermore, the updated transcriptomic and proteomic approaches have been employed to PA function research; however, the precise roles of PAs remain largely elusive. With the recent reestablishment of the roles of reactive oxygen species (ROS) in the stress response and the relationship between the nitric oxide biosynthesis and PA titers, the roles of PA in the stress response are attracting more attention than ever, which provides a good chance to make a retrospect to the past studies on the relationships between PAs and stresses, including biotic and abiotic stresses (Pang *et al.*, 2007).

Polyamines (PAs) are ubiquitous low-molecular-weight aliphatic amines that are involved in regulation of plant growth and development. PAs are also implicated in a wide range of environmental stress tolerance in plants. New roles are being discovered every day for these interesting molecules in

the plant world. In higher plants, the most common PAs are spermidine (Spd) their diamine obligate precursor putrescine (Put). Like PAs displaying high biological activity are involved in a wide array of fundamental processes in plants, such as replication and gene expression, growth and development, senescence, membrane stabilization, enzyme activity modulation and adaptation to abiotic stresses (Alet *et al.*, 2012). However, the precise physiological function and mechanism of action of PAs still remain unclear. In contrast to the reliable works on the role of PAs in plants defense against biotic and abiotic stresses, few reports recently indicated that PAs may act as cellular signals in intrinsic talk with hormonal pathways including ABA (Alcazar *et al.*, 2010; Gill and Tuteja, 2010). Changes in plant PA metabolism occur in response to a variety of abiotic stresses (Alcazar *et al.*, 2006; Gill and Tuteja, 2010). These changes in cellular PA under stress only provide clues on its possible implication in stress response, but they do not provide evidence of its role in counteracting stress. Hence, to understand whether PA actually protect cells from stress induced damages, exogenous application of PA, which is expected to increase endogenous PA, has been investigated before or during stress (Wang *et al.*, 2007). It has been reported that exogenous application of PAs could alleviate salt induced reduction in photosynthetic efficiency, but this effect is strongly dependent both on PAs concentration or types and stress levels (Duan *et al.*, 2008). The result obtained by Zhang *et al.*, (2009) suggested that Put strongly affects photosynthetic apparatus involving in enhancement of photochemical quenching rather than regulation of stomatal closure or opening. Several publications have reported that changes of endogenous PA level and forms are involved in regulating the photochemical efficiency of salt-stressed plants, and PAs metabolism-related enzymes are closely correlated with photosynthesis.

Therefore overall aim of this study is to elucidate the polyamine induced physiological and biochemical changes responsible for induction of sodicity tolerance in sugarcane.

Materials and Methods

Experimental details

The sugarcane sets of a salt tolerant *viz.*, CoM 0265 and a salt susceptible CoC 671 were obtained from CSRS Padegaon. The sets with an eye bud were washed and sterilized with sodium hypochlorite (0.1% w/v). The sets were dipped in the solution of individual polyamines *viz.*, 0, 100 and 500 μ M of putrescine and spermidine. These sets were planted in sodic soil and normal soil in triplicate. The two foliar applications of these polyamines were carried out 45 days after planting with 8 days interval. The leaf samples were collected after 65 days after planting for the biochemical analyses.

Chlorophyll a and b content and Total chlorophyll, by Arnon, (1949)

The leaf samples of sugarcane were cut into small pieces and known weight (0.2 g) of fresh leaf sample was macerated in a mortar and pestle and extracted with 20 mL of 80 per cent acetone. The content was centrifuged at 5000xg for 10 min and the supernatant was collected. The final volume of extract was made to 50 mL. The extinction of chlorophyll extract was recorded at 645 and 663 nm on a Spectrophotometer and a blank was run with 80 per cent acetone.

Lipid peroxidation by Heath and Packer, (1968)

Sample extraction: Leaf sample 0.2 g was homogenized in 4 mL of 0.1 % TCA. The homogenate was centrifuged at 15000 g for 15 min and the supernatant was used for the

estimation of TBARS content. Four mL of 0.5 % TBA in 20 % TCA was added in 10 mL aliquot of the supernatant. The mixture was heated at 95°C for 30 min in the lab electric oven and then cooled in an ice bath.

After cooling the aliquot was centrifuged at 10,000 Xg for 10 min. The absorbance of the clear supernatant was recorded at 532 nm. Values of nonspecific absorption recorded at 600 nm and were subtracted from the values recorded at 532 nm. The TBARS content was calculated by using extinction coefficient $E = 155$ nmoles of MDA formed $\text{mg}^{-1}\text{fr. wt.}$

Reducing sugars by Nelson-Somogyi (1944)

0.2 mL of sugar extract and 0.8 mL of distilled water, one mL of copper reagent was added. The content were mixed and heated for 20 min. in boiling water bath. The tubes were cooled. One mL of arsenomolybdate reagent was added and mixed well. This mixture was diluted with 7 mL of distilled water and the absorbance was taken at 520 nm against blank.

The reducing were calculated from a standard curve for D-glucose and expressed as milligrams per 100 g dry weight.

Total sugars by Nelson-Somogyi (1944)

To the 25 mL of ethanolic extract 2.5 mL of hydrochloric acid was added and kept for 24 hours for inversion. It was neutralized with 0.1N sodium hydroxide using pH meter and the volume was made to 50 mL. The hydrolyzed sugars were estimated as total sugars from the leaves of sugarcane grown in normal and sodic soil.

Non-Reducing sugars by Nelson-Somogyi (1944)

Non-reducing sugars content were calculated as difference between total and reducing sugars.

Results and Discussion

Chlorophyll 'a'

Chlorophyll 'a' content was decreased in CoC 671 a salt susceptible cultivar and CoM 0265 a salt tolerant cultivar when grown in sodic soil (Table 1). However, with the application of polyamines chlorophyll 'a' of sugarcane grown under normal and sodic soil was increased with increase in the polyamine concentration in both the cultivar (Table 1). The chlorophyll 'a' of the variety CoM 0265 by the application of putrescine and spermidine was 0.76 mg g^{-1} ($0\mu\text{M}$), 0.91 mg g^{-1} ($100\mu\text{M}$), 1.03 mg g^{-1} ($500\mu\text{M}$) and 0.74 mg g^{-1} ($0\mu\text{M}$), 0.87 mg g^{-1} ($100\mu\text{M}$), 0.97 mg g^{-1} ($500\mu\text{M}$) respectively in normal soil. The chlorophyll 'a' of the variety CoM 0265 with the application of putrescine and spermidine was 0.49 mg g^{-1} ($0\mu\text{M}$), 1.20 mg g^{-1} ($100\mu\text{M}$), 1.97 mg g^{-1} ($500\mu\text{M}$) and 0.47 mg g^{-1} ($0\mu\text{M}$), 0.96 mg g^{-1} ($100\mu\text{M}$), 1.49 mg g^{-1} ($500\mu\text{M}$) respectively in sodic soil. The chlorophyll 'a' of the variety CoC 671 by the application of putrescine and spermidine was 0.72 mg g^{-1} ($0\mu\text{M}$), 0.89 mg g^{-1} ($100\mu\text{M}$), 1.03 mg g^{-1} ($500\mu\text{M}$) and 0.70 mg g^{-1} ($0\mu\text{M}$), 0.85 mg g^{-1} ($100\mu\text{M}$), 0.99 mg g^{-1} ($500\mu\text{M}$) respectively in normal soil.

The chlorophyll 'a' of the variety CoC 671 by the application of putrescine and spermidine was 0.41 mg g^{-1} ($0\mu\text{M}$), 1.23 mg g^{-1} ($100\mu\text{M}$), 1.94 mg g^{-1} ($500\mu\text{M}$) and 0.43 mg g^{-1} ($0\mu\text{M}$), 1.16 mg g^{-1} ($100\mu\text{M}$), 1.85 mg g^{-1} ($500\mu\text{M}$) respectively in sodic soil.

Chlorophyll 'b'

From Table 2 it was noticed that chlorophyll 'b' in sugarcane varieties CoM 0265 salt tolerant and CoC 671 salt susceptible was reduced when grown in sodic soil. Effect of application of polyamine on chlorophyll 'b' of sugarcane grown under normal and sodic soil revealed that there was increase in chlorophyll

'b' with increase in the polyamine concentration under normal and sodic soil in both on the cultivar (Table 2). The chlorophyll 'b' of the variety CoM 0265 by the application of putrescine and spermidine was 0.54 mg g^{-1} ($0 \mu\text{M}$), 0.69 mg g^{-1} ($100 \mu\text{M}$), 0.81 mg g^{-1} ($500 \mu\text{M}$) and 0.74 mg g^{-1} ($0 \mu\text{M}$), 0.87 mg g^{-1} ($100 \mu\text{M}$), 0.97 mg g^{-1} ($500 \mu\text{M}$) respectively in normal soil.

The chlorophyll 'b' of the variety CoM 0265 by the application of putrescine and spermidine was 0.47 mg g^{-1} ($0 \mu\text{M}$), 1.18 mg g^{-1} ($100 \mu\text{M}$), 1.95 mg g^{-1} ($500 \mu\text{M}$) and 0.47 mg g^{-1} ($0 \mu\text{M}$), 0.96 mg g^{-1} ($100 \mu\text{M}$), 1.49 mg g^{-1} ($500 \mu\text{M}$) respectively in sodic soil. The chlorophyll 'b' of the variety CoC 671 by the application of putrescine and spermidine was 0.46 mg g^{-1} ($0 \mu\text{M}$), 0.63 mg g^{-1} ($100 \mu\text{M}$), 0.77 mg g^{-1} ($500 \mu\text{M}$) and 0.46 mg g^{-1} ($0 \mu\text{M}$), 0.61 mg g^{-1} ($100 \mu\text{M}$), 0.75 mg g^{-1} ($500 \mu\text{M}$) respectively in normal soil. The chlorophyll 'b' of the variety CoC 671 by the application of putrescine and spermidine was 0.41 mg g^{-1} ($0 \mu\text{M}$), 1.23 mg g^{-1} ($100 \mu\text{M}$), 1.94 mg g^{-1} ($500 \mu\text{M}$) and 0.43 mg g^{-1} ($0 \mu\text{M}$), 1.16 mg g^{-1} ($100 \mu\text{M}$), 1.85 mg g^{-1} ($500 \mu\text{M}$) in sodic soil.

Total chlorophyll

The total chlorophyll in leaves of sugarcane varieties viz. CoM 0265 a salt tolerant and CoC 671 a salt susceptible were decreased when planted in sodic soil (Table 3). However, the application of polyamine on total chlorophyll of sugarcane grown under normal and sodic soil the revealed that it was increased is decreased with increase in the polyamine concentrations content in both on the cultivar (Table 3). The total chlorophyll content in the variety CoM 265 by the application of putrescine and spermidine was 1.30 mg g^{-1} ($0 \mu\text{M}$), 1.60 mg g^{-1} ($100 \mu\text{M}$), 1.84 mg g^{-1} ($500 \mu\text{M}$) and 1.26 mg g^{-1} ($0 \mu\text{M}$), 1.52 mg g^{-1} ($100 \mu\text{M}$), 1.72 mg g^{-1} ($500 \mu\text{M}$) respectively in normal soil.

Lipid peroxidation

Lipid peroxidation was reduced in the sugarcane varieties viz., CoM 0265, salt tolerant and CoC 671, salt susceptible grown in sodic soil when treated with polyamines (Table 4). Effect of polyamine on lipid peroxidation of sugarcane varieties grown under normal and sodic soil revealed that with increase in the polyamine concentration under normal and sodic soil in both on the cultivar there was reduction of lipid peroxidation rate (Table 4). The lipid peroxidation of the variety CoM 0265 at concentration of putrescine and spermidine was $28.03 \text{ } \mu\text{moles of MDA formed mg}^{-1} \text{ fr.wt.}$ ($0 \mu\text{M}$), $28.00 \text{ } \mu\text{moles of MDA formed mg}^{-1} \text{ fr.wt.}$ ($100 \mu\text{M}$), $27.99 \text{ } \mu\text{moles of MDA formed mg}^{-1} \text{ fr.wt.}$ ($500 \mu\text{M}$) and $28.00 \text{ } \mu\text{moles of MDA formed mg}^{-1} \text{ fr.wt.}$ ($0 \mu\text{M}$), $28.16 \text{ } \mu\text{moles of MDA formed mg}^{-1} \text{ fr.wt.}$ ($100 \mu\text{M}$), $28.17 \text{ } \mu\text{moles of MDA formed mg}^{-1} \text{ fr.wt.}$ ($500 \mu\text{M}$) in normal soil.

The lipid peroxidation of the variety CoM 0265 at concentration of putrescine and spermidine was $28.33 \text{ } \mu\text{moles of MDA formed mg}^{-1} \text{ fr.wt.}$ ($0 \mu\text{M}$), $28.19 \text{ } \mu\text{moles of MDA formed mg}^{-1} \text{ fr.wt.}$ ($100 \mu\text{M}$), $28.10 \text{ } \mu\text{moles of MDA formed mg}^{-1} \text{ fr.wt.}$ ($500 \mu\text{M}$) and $28.31 \text{ } \mu\text{moles of MDA formed mg}^{-1} \text{ fr.wt.}$ ($0 \mu\text{M}$), $28.21 \text{ } \mu\text{moles of MDA formed mg}^{-1} \text{ fr.wt.}$ ($100 \mu\text{M}$), $28.16 \text{ } \mu\text{moles of MDA formed mg}^{-1} \text{ fr.wt.}$ ($500 \mu\text{M}$) in sodic soil.

The lipid peroxidation of the variety CoC 671 at concentration of putrescine and spermidine was $18.55 \text{ } \mu\text{moles of MDA formed mg}^{-1} \text{ fr.wt.}$ ($0 \mu\text{M}$), $18.50 \text{ } \mu\text{moles of MDA formed mg}^{-1} \text{ fr.wt.}$ ($100 \mu\text{M}$), $18.48 \text{ } \mu\text{moles of MDA formed mg}^{-1} \text{ fr.wt.}$ ($500 \mu\text{M}$) and $18.41 \text{ } \mu\text{moles of MDA formed mg}^{-1} \text{ fr.wt.}$ ($0 \mu\text{M}$), $18.40 \text{ } \mu\text{moles of MDA formed mg}^{-1} \text{ fr.wt.}$ ($100 \mu\text{M}$), $18.38 \text{ } \mu\text{moles of MDA formed mg}^{-1} \text{ fr.wt.}$ ($500 \mu\text{M}$) in normal soil.

Table.1 Effect of polyamines on chlorophyll a in sugarcane leaves grown on normal and sodic soils

Sr. No.	Name of the cultivar/ Name of the polyamine	Chlorophyll a (mg g ⁻¹)						Fold Increase (+) / Fold Decrease (-)
		Normal soil			Sodic soil			
		Concentration of the polyamine (µM)						
		0	100	500	0	100	500	
1.	CoM 265 (Salt tolerant cultivar)							
a.	Putrescine	0.76 (00)	1.20 (+1.58)	1.97 (+2.59)	0.49 (-0.64)	0.91 (+1.19)	1.03 (+1.35)	Over the control of normal soil
		-	-	-	(00)	(+1.88)	(+2.10)	Over the control of sodic soil
		-	-	-	-	(+0.76)	(-0.52)	Over respective Putrescine conc.
b.	Spermidine	0.74 (00)	0.96 (+1.30)	1.49 (+2.01)	0.47 (-0.63)	0.87 (+1.18)	0.97 (+1.31)	Over the control of normal soil
		-	-	-	(00)	(+1.85)	(+2.06)	Over the control of sodic soil
		-	-	-	-	(-0.97)	(+0.65)	Over respective Spermidine conc
2.	CoC 671 (Salt susceptible cultivar)							
a.	Putrescine	0.72 (00)	1.23 (+1.71)	1.94 (+2.70)	0.41 (-0.57)	0.89 (+1.24)	1.03 (+1.43)	Over the control of normal soil
		-	-	-	(00)	(+2.17)	(+2.51)	Over the control of sodic soil
		-	-	-	-	(-0.72)	(-0.53)	Over respective Putrescine conc.
b.	Spermidine	0.70 (00)	1.16 (+1.66)	1.85 (+2.64)	0.43 (-0.62)	0.85 (+1.22)	0.99 (+1.42)	Over the control of normal soil
		-	-	-	(00)	(+1.98)	(+2.30)	Over the control of sodic soil
		-	-	-	-	(-0.73)	(-0.54)	Over respective Spermidine conc
	Comparison	S.Em. ±	CD at 5%		Comparison	S.Em. ±	CD at 5%	
	Variety (V)	0.004	0.012		T*L	0.007	0.021	
	Soil (S)	0.004	0.012		T*S	0.006	0.017	
	Type of polyamine (T)	0.004	0.012		V*S*T	0.008	0.025	
	Conc. of Polyamines (L)	0.005	0.015		V*T*L	0.010	0.030	
	V*S	0.006	0.017		S*T*L	0.010	0.030	
	V*T	0.006	0.017		V*S*L	0.010	0.030	
					V*S*T*L	0.015	0.043	

Table.2 Effect of polyamines on chlorophyll b in sugarcane leaves grown on normal and sodic soils

Sr. No.	Name of the cultivar/ Name of the polyamine	Chlorophyll b (mg g ⁻¹)						Fold Increase (+) / Fold Decrease (-)
		Normal soil			Sodic soil			
		Concentration of the polyamine (μM)						
		0	100	500	0	100	500	
1.	CoM 265 (Salt tolerant cultivar)							
a.	Putrescine	0.54 (00)	0.69 (+1.28)	0.81 (+1.5)	0.47 (-0.87)	1.18 (+2.18)	1.95 (+3.61)	Over the control of normal soil
		-	-	-	(00)	(+2.51)	(+4.15)	Over the control of sodic soil
		-	-	-	-	(+1.71)	(+2.41)	Over respective Putrescine conc.
b.	Spermidine	0.74 (00)	0.87 (+1.17)	0.97 (+1.31)	0.47 (-0.63)	0.96 (+1.30)	1.49 (+2.01)	Over the control of normal soil
		-	-	-	(00)	(+2.04)	(+3.17)	Over the control of sodic soil
		-	-	-	-	(+1.10)	(+1.54)	Over respective Spermidine conc
2.	CoC 671 (Salt susceptible cultivar)							
a.	Putrescine	0.46 (00)	0.63 (+1.37)	0.77 (+1.67)	0.41 (-0.89)	1.23 (+2.67)	1.94 (+4.21)	Over the control of normal soil
		-	-	-	(00)	(+3)	(+4.73)	Over the control of sodic soil
		-	-	-	-	(+1.95)	(+2.51)	Over respective Putrescine conc.
b.	Spermidine	0.46 (00)	0.61 (+1.32)	0.75 (+1.63)	0.43 (-0.93)	1.16 (+2.52)	1.85 (+4.02)	Over the control of normal soil
		-	-	-	(00)	(+2.69)	(+4.30)	Over the control of sodic soil
		-	-	-	-	(+1.90)	(+2.46)	Over respective Spermidine conc
	Comparison	S.Em. ±	CD at 5%		Comparison	S.Em. ±	CD at 5%	
	Variety (V)	0.005	0.014		T*L	0.008	0.024	
	Soil (S)	0.005	0.014		T*S	0.007	0.019	
	Type of polyamine (T)	0.005	0.014		V*S*T	0.009	0.028	
	Conc. of Polyamines (L)	0.006	0.017		V*T*L	0.012	0.034	
	V*S	0.007	0.019		S*T*L	0.012	0.034	
	V*T	0.007	0.019		V*S*L	0.012	0.034	
					V*S*T*L	0.017	0.048	

Table.3 Effect of polyamines on total chlorophyll in sugarcane leaves grown on normal and sodic soils

Sr. No .	Name of the cultivar/ Name of the polyamine	Total Chlorophyll (mg g ⁻¹)						Fold Increase (+) / Fold Decrease (-)
		Normal soil			Sodic soil			
		Concentration of the polyamine (µM)						
		0	100	500	0	100	500	
1.	CoM 265 (Salt tolerant cultivar)							
a.	Putrescine	1.30 (00)	1.60 (+1.23)	1.84 (+1.42)	0.96 (-0.73)	2.38 (+1.83)	3.92 (+3.02)	Over the control of normal soil
		-	-	-	(00)	(+2.47)	(+4.08)	Over the control of sodic soil
		-	-	-	-	(+1.48)	(+2.13)	Over respective Putrescine conc.
b.	Spermidine	1.26 (00)	1.52 (+1.20)	1.72 (+1.37)	0.94 (-0.74)	1.92 (+1.52)	2.98 (+2.37)	Over the control of normal soil
		-	-	-	(00)	(+2.04)	(+3.17)	Over the control of sodic soil
		-	-	-	-	(+1.26)	(+1.73)	Over respective Spermidine conc
2.	CoC 671 (Salt susceptible cultivar)							
a.	Putrescine	1.18 (00)	1.52 (+1.28)	1.80 (+1.53)	0.82 (-0.69)	2.46 (+2.08)	3.88 (+3.28)	Over the control of normal soil
		-	-	-	(00)	(+3)	(+4.73)	Over the control of sodic soil
		-	-	-	-	(+1.61)	(+2.16)	Over respective Putrescine conc.
b.	Spermidine	1.16 (00)	1.46 (+1.25)	1.74 (+1.5)	0.86 (-0.74)	2.32 (+2)	3.70 (+3.18)	Over the control of normal soil
		-	-	-	(00)	(+2.69)	(+4.30)	Over the control of sodic soil
		-	-	-	-	(+1.58)	(+2.12)	Over respective Spermidine conc
	Comparison	S.Em. ±	CD at 5%		Comparison	S.Em. ±	CD at 5%	
	Variety (V)	0.019	0.05		T*L	0.033	0.09	
	Soil (S)	0.019	NS		T*S	0.027	NS	
	Type of polyamine (T)	0.019	0.05		V*S*T	0.038	0.11	
	Conc. of Polyamines (L)	0.023	0.06		V*T*L	0.047	0.13	
	V*S	0.027	NS		S*T*L	0.047	0.13	
	V*T	0.027	0.07		V*S*L	0.047	0.13	
					V*S*T*L	0.067	0.19	

Table.4 Effect of polyamines on lipid peroxidation in sugarcane leaves grown on normal and sodic soils

Sr. No.	Name of the cultivar/ Name of the polyamine	Lipid peroxidation (ηmoles of MDA formed mg ⁻¹ fr.wt.)						Fold Increase (+) / Fold Decrease (-)
		Normal soil			Sodic soil			
		Concentration of the polyamine (μM)						
		0	100	500	0	100	500	
1.	CoM 265 (Salt tolerant cultivar)							
a.	Putrescine	28.03 (00)	28.00 (-0.99)	27.99 (-0.99)	28.03 (1)	28.19 (+1.00)	28.10 (+1.00)	Over the control of normal soil
		-	-	-	(00)	(+1.00)	(+1.00)	Over the control of sodic soil
		-	-	-	-	(+1.00)	(+1.00)	Over respective Putrescine conc.
b.	Spermidine	28.00 (00)	28.16 (+1.00)	28.17 (+1.00)	28.31 (+1.01)	28.16 (+1.00)	28.17 (1.00)	Over the control of normal soil
		-	-	-	(00)	(-0.99)	(-0.99)	Over the control of sodic soil
		-	-	-	-	(1)	(1)	Over respective Spermidine conc
2.	CoC 671 (Salt susceptible cultivar)							
a.	Putrescine	18.55 (00)	18.50 (-0.99)	18.48 (-0.99)	21.07 (+1.13)	19.44 (+1.04)	18.88 (+1.01)	Over the control of normal soil
		-	-	-	(00)	(-0.92)	(-0.89)	Over the control of sodic soil
		-	-	-	-	(+1.05)	(+1.02)	Over respective Putrescine conc.
b.	Spermidine	18.41 (00)	18.40 (+0.99)	18.38 (-0.99)	21.13 (1.14)	19.15 (+1.04)	18.66 (+1.01)	Over the control of normal soil
		-	-	-	(00)	(-0.90)	(-0.88)	Over the control of sodic soil
		-	-	-	-	(+1.04)	(+1.02)	Over respective Spermidine conc
	Comparison	S.Em. ±	CD at 5%		Comparison	S.Em. ±	CD at 5%	
	Variety (V)	0.098	0.28		T*L	0.181	0.51	
	Soil (S)	0.100	0.28		T*S	0.145	0.55	
	Type of polyamine (T)	0.100	0.28		V*S*T	0.197	0.56	
	Conc. of Polyamines (L)	0.124	0.35		V*T*L	0.242	0.68	
	V*S	0.597	1.69		S*T*L	0.270	0.76	
	V*T	0.597	1.69		V*S*L	0.242	0.68	
					V*S*T*L	0.342	NS	

Table.5 Effect of polyamines on reducing sugars in sugarcane leaves grown on normal and sodic soils

Sr. No.	Name of the cultivar/ Name of the polyamine	Reducing sugars (mg g ⁻¹ DW)						Fold Increase (+) / Fold Decrease (-)
		Normal soil			Sodic soil			
		Concentration of the polyamine (μM)						
		0	100	500	0	100	500	
1.	CoM 265 (Salt tolerant cultivar)							
a.	Putrescine	3.33 (00)	3.48 (+1.04)	3.66 (+1.09)	2.89 (-0.86)	3.14 (-0.94)	3.52 (+1.05)	Over the control of normal soil
		-	-	-	(00)	(+1.08)	(+1.21)	Over the control of sodic soil
						(-0.90)	(-0.96)	Over respective Putrescine conc.
b.	Spermidine	3.00 (00)	3.15 (+1.05)	3.27 (+1.09)	2.90 (+0.96)	3.01 (+1.00)	3.22 (+1.07)	Over the control of normal soil
		-	-	-	(00)	(+1.03)	(+1.10)	Over the control of sodic soil
		-	-	-	-	(-0.96)	(-0.98)	Over respective Spermidine conc
2.	CoC 671 (Salt susceptible cultivar)							
a.	Putrescine	2.03 (00)	2.26 (+1.11)	2.60 (+1.28)	1.78 (+0.87)	2.57 (+1.26)	3.26 (+1.60)	Over the control of normal soil
		-	-	-	(00)	(+1.44)	(+1.83)	Over the control of sodic soil
		-	-	-	-	(+1.13)	(+1.25)	Over respective Putrescine conc.
b.	Spermidine	2.00 (00)	2.15 (+1.08)	2.29 (+1.15)	1.80 (+0.9)	2.14 (+1.07)	2.45 (+1.23)	Over the control of normal soil
		-	-	-	(00)	(+1.18)	(+1.36)	Over the control of sodic soil
		-	-	-	-	(-0.100)	(+1.06)	Over respective Spermidine conc
	Comparison	S.Em. ±	CD at 5%		Comparison	S.Em. ±	CD at 5%	
	Variety (V)	0.006	0.017		T*L	0.010	0.030	
	Soil (S)	0.006	0.017		T*S	0.008	0.024	
	Type of polyamine (T)	0.006	0.017		V*S*T	0.012	0.034	
	Conc. of Polyamines (L)	0.007	0.021		V*T*L	0.015	0.042	
	V*S	0.008	0.024		S*T*L	0.015	0.042	
	V*T	0.008	0.024		V*S*L	0.015	0.042	
					V*S*T*L	0.021	0.060	

Table.6 Effect of polyamines on non-reducing sugars in sugarcane leaves grown on normal and sodic soils

Sr. No.	Name of the cultivar/ Name of the polyamine	Non-Reducing sugars (mg g ⁻¹ DW)						Fold Increase (+) / Fold Decrease (-)
		Normal soil			Sodic soil			
		Concentration of the polyamine (μM)						
		0	100	500	0	100	500	
1.	CoM 265 (Salt tolerant cultivar)							
a.	Putrescine	4.25 (00)	4.39 (+1.03)	4.56 (+1.07)	3.55 (-0.83)	3.80 (-0.89)	4.18 (+0.98)	Over the control of normal soil
		-	-	-	(00)	(+1.07)	(+1.17)	Over the control of sodic soil
		-	-	-	-	(-0.87)	(-0.91)	Over respective Putrescine conc.
b.	Spermidine	4.26 (00)	4.41 (+1.03)	4.53 (+1.06)	3.56 (-0.84)	3.67 (-0.86)	3.88 (+0.91)	Over the control of normal soil
		-	-	-	(00)	(+1.03)	(+1.08)	Over the control of sodic soil
		-	-	-	-	(-0.83)	(-0.85)	Over respective Spermidine conc
2.	CoC 671 (Salt susceptible cultivar)							
a.	Putrescine	3.36 (00)	3.59 (+1.06)	3.93 (+1.16)	1.89 (-0.56)	2.68 (-0.79)	3.37 (+1.00)	Over the control of normal soil
		-	-	-	(00)	(+1.41)	(+1.78)	Over the control of sodic soil
						(-0.91)	(-0.85)	Over respective Putrescine conc.
b.	Spermidine	3.33 (00)	3.48 (+1.04)	3.62 (+1.08)	1.80 (-0.54)	2.14 (-0.64)	2.45 (-0.73)	Over the control of normal soil
		-	-	-	(00)	(+1.18)	(+1.36)	Over the control of sodic soil
		-	-	-	-	(-0.61)	(-0.67)	Over respective Spermidine conc
	Comparison	S.Em. ±	CD at 5%		Comparison	S.Em. ±	CD at 5%	
	Variety (V)	0.007	0.021		T*L	0.013	0.038	
	Soil (S)	0.007	0.021		T*S	0.010	0.030	
	Type of polyamine (T)	0.007	0.021		V*S*T	0.015	0.043	
	Conc. of Polyamines (L)	0.009	0.026		V*T*L	0.018	0.053	
	V*S	0.010	0.030		S*T*L	0.018	0.053	
	V*T	0.010	0.030		V*S*L	0.018	0.053	
					V*S*T*L	0.026	0.075	

Table.7 Effect of polyamines on total sugars in sugarcane leaves grown on normal and sodic soils

Sr. No.	Name of the cultivar/ Name of the polyamine	Total sugars (mg g ⁻¹ DW)						Fold Increase (+) / Fold Decrease (-)
		Normal soil			Sodic soil			
		Concentration of the polyamine (μM)						
		0	100	500	0	100	500	
1.	CoM 265 (Salt tolerant cultivar)							
a.	Putrescine	7.58 (00)	7.87 (+1.03)	8.22 (+1.08)	6.44 (-0.84)	6.94 (-0.91)	7.70 (+1.02)	Over the control of normal soil Over the control of sodic soil Over respective Putrescine conc.
		-	-	-	(00)	(+1.10)	(+1.20)	
		-	-	-	-	(-0.88)	(-0.93)	
b.	Spermidine	7.26 (00)	7.56 (+1.04)	7.80 (+1.07)	6.46 (-0.88)	6.68 (-0.92)	7.10 (-0.97)	Over the control of normal soil Over the control of sodic soil Over respective Spermidine conc
		-	-	-	(00)	(+1.03)	(+1.09)	
		-	-	-	-	(-0.88)	(-0.91)	
2.	CoC 671 (Salt susceptible cultivar)							
a.	Putrescine	5.39 (00)	5.85 (+1.09)	6.53 (+1.21)	3.67 (-0.68)	5.25 (-0.97)	6.63 (+1.23)	Over the control of normal soil Over the control of sodic soil Over respective Putrescine conc.
		-	-	-	(00)	(+1.43)	(+1.80)	
		-	-	-	-	(-0.89)	(+1.02)	
b.	Spermidine	5.33 (00)	5.63 (+1.05)	5.91 (+1.10)	3.60 (-0.68)	4.28 (-0.80)	4.90 (-0.91)	Over the control of normal soil Over the control of sodic soil Over respective Spermidine conc
		-	-	-	(00)	(+1.18)	(+1.36)	
		-	-	-	-	(-0.76)	(-0.82)	
	Comparison	S.Em. ±	CD at 5%		Comparison	S.Em. ±	CD at 5%	
	Variety (V)	0.047	0.13		T*L	0.082	0.23	
	Soil (S)	0.047	0.13		T*S	0.067	0.19	
	Type of polyamine (T)	0.047	0.13		V*S*T	0.094	0.26	
	Conc. of Polyamines (L)	0.058	0.16		V*T*L	0.116	0.32	
	V*S	0.067	NS		S*T*L	0.116	0.32	
	V*T	0.067	0.19		V*S*L	0.116	0.32	
					V*S*T*L	0.164	0.46	

The lipid peroxidation of the variety CoC 671 at concentration of putrescine and spermidine was 21.07ηmoles of MDA formed mg⁻¹ fr.wt. (0μM), 19.44ηmoles of MDA formed mg⁻¹ fr.wt. (100μM), 18.88ηmoles of MDA formed mg⁻¹ fr.wt. (500μM) and 21.13ηmoles of

MDA formed mg^{-1} fr.wt. ($0\mu\text{M}$), $19.15\eta\text{moles}$ of MDA formed mg^{-1} fr.wt. ($100\mu\text{M}$), $18.66\eta\text{moles}$ of MDA formed mg^{-1} fr.wt. ($500\mu\text{M}$) in sodic soil.

Hosseini *et al.*, (2010) observed that MDA level increased gradually in two varieties of sugarcane by NaCl stress. Similar trend was observed in the present study due to sodicity. Similar trend was also reported by Pagariya *et al.*, (2012) in sugarcane leaves. The membrane damage is caused by lipid peroxidation resulting from the uncontrolled ROS production (Rodriguez-Rosales *et al.*, 1999). Hala *et al.*, (2005) reported that salt stress decreased the PUT level in two wheat cultivars with increase in salinity level. However, Spd and Spm were increased with increase in the salinity levels in two wheat cultivars. Erdei *et al.*, (1996) indicated that Spd and Spm accumulation is one of the adaptive responses to salt stress. In the present investigation application PAs resulted in lowering lipid peroxidation in sugarcane cultivars.

Reducing sugar

The reducing sugar in the leaves of two sugarcane varieties *viz.*, CoM 0265 and CoC 671 were decreased when grown in sodic soil (Table 5). The effect of polyamines on reducing sugars in sugarcane grown under normal and sodic soil the resulted in increased in reducing sugar with increase in the polyamine concentration in both on the cultivar (Table 5).

The reducing sugar of the variety CoM 0265 at concentration of putrescine and spermidine was 3.33mg g^{-1} ($0\mu\text{M}$), 3.48mg g^{-1} ($100\mu\text{M}$), 3.66mg g^{-1} ($500\mu\text{M}$) and 3.00mg g^{-1} ($0\mu\text{M}$), 3.15mg g^{-1} ($100\mu\text{M}$), 3.27mg g^{-1} ($500\mu\text{M}$) in normal soil. The reducing sugar of the variety CoM 0265 at concentration of putrescine and spermidine was 2.89mg g^{-1} ($0\mu\text{M}$), 3.14mg g^{-1} ($100\mu\text{M}$), 3.52mg g^{-1} ($500\mu\text{M}$) and 2.90mg g^{-1}

($0\mu\text{M}$), 3.01mg g^{-1} ($100\mu\text{M}$), 3.22mg g^{-1} ($500\mu\text{M}$) in sodic soil. The reducing sugar of the variety CoC 671 at concentration of putrescine and spermidine was 2.03mg g^{-1} ($0\mu\text{M}$), 2.26mg g^{-1} ($100\mu\text{M}$), 2.60mg g^{-1} ($500\mu\text{M}$) and 2.00mg g^{-1} ($0\mu\text{M}$), 2.15mg g^{-1} ($100\mu\text{M}$), 2.29mg g^{-1} ($500\mu\text{M}$) in normal soil. The reducing sugar of the variety CoC 671 at concentration of putrescine and spermidine was 1.78mg g^{-1} ($0\mu\text{M}$), 2.57mg g^{-1} ($100\mu\text{M}$), 3.26mg g^{-1} ($500\mu\text{M}$) and 1.80mg g^{-1} ($0\mu\text{M}$), 2.14mg g^{-1} ($100\mu\text{M}$), 2.45mg g^{-1} ($500\mu\text{M}$) in sodic soil.

Non reducing sugar

The sugarcane varieties, CoM 0265 and CoC 671 grown in sodic soil caused the reduction in the reducing sugars (Table 6). The effect of polyamines on non reducing sugars of sugarcane leaves grown under normal and sodic soil resulted increased with increase in the polyamine concentration in both on the cultivar (Table 6). The non reducing sugar in the variety CoM 0265 at concentration of putrescine and spermidine was 4.25mg g^{-1} ($0\mu\text{M}$), 4.39mg g^{-1} ($100\mu\text{M}$), 4.56mg g^{-1} ($500\mu\text{M}$) and 4.26mg g^{-1} ($0\mu\text{M}$), 4.41mg g^{-1} ($100\mu\text{M}$), 4.53mg g^{-1} ($500\mu\text{M}$) in normal soil. The non-reducing sugar of the variety CoM 0265 at concentration of putrescine and spermidine was 3.55mg g^{-1} ($0\mu\text{M}$), 3.80mg g^{-1} ($100\mu\text{M}$), 4.18mg g^{-1} ($500\mu\text{M}$) and 3.56mg g^{-1} ($0\mu\text{M}$), 3.67mg g^{-1} ($100\mu\text{M}$), 3.88mg g^{-1} ($500\mu\text{M}$) in sodic soil. The non-reducing sugar of the variety CoC 671 at concentration of putrescine and spermidine was 3.36mg g^{-1} ($0\mu\text{M}$), 3.59mg g^{-1} ($100\mu\text{M}$), 3.93mg g^{-1} ($500\mu\text{M}$) and 3.33mg g^{-1} ($0\mu\text{M}$), 3.48mg g^{-1} ($100\mu\text{M}$), 3.62mg g^{-1} ($500\mu\text{M}$) in normal soil. The non-reducing sugar of the variety CoC 671 at concentration of putrescine and spermidine was 1.89mg g^{-1} ($0\mu\text{M}$), 2.68mg g^{-1} ($100\mu\text{M}$), 3.37mg g^{-1} ($500\mu\text{M}$) and 1.80mg g^{-1} ($0\mu\text{M}$), 2.14mg g^{-1} ($100\mu\text{M}$), 2.45mg g^{-1} ($500\mu\text{M}$) in sodic soil.

Total sugar

The total sugar was reduced in sugarcane varieties CoM 0265 and CoC 671 when grown in the sodic soil (Table 7). However the application of polyamines on sugarcane plant grown under normal and sodic soil resulted increased in total sugar with increase in the polyamine concentration in both on the cultivar (Table 7). The total sugar of the variety CoM 0265 at concentration of putrescine and spermidine was 7.58mg g⁻¹ (0μM), 7.87mg g⁻¹ (100μM), 8.22 mg g⁻¹ (500μM) and 7.26mg g⁻¹ (0μM), 7.56mg g⁻¹ (100μM), 7.80mg g⁻¹ (500μM) in normal soil. The total sugar of the variety CoM 0265 at concentration of putrescine and spermidine was 6.44mg g⁻¹ (0μM), 6.94 mg g⁻¹(100μM), 7.70 mg g⁻¹(500μM) and 6.46mg g⁻¹ (0μM), 6.68mg g⁻¹ (100μM), 7.20mg g⁻¹(500μM) in sodic soil. The total sugar of the variety CoC 671 at concentration of putrescine and spermidine was 5.39mg g⁻¹ (0μM), 5.85mg g⁻¹ (100μM), 6.53mg g⁻¹ (500μM) and 5.33mg g⁻¹ (0μM), 5.63mg g⁻¹ (100μM), 5.91mg g⁻¹ (500μM) in normal soil. The total sugar of the variety CoC 671 at concentration of putrescine and spermidine was 3.67mg g⁻¹ (0μM), 5.25mg g⁻¹ (100μM), 6.63mg g⁻¹ (500μM) and 3.60mg g⁻¹(0μM), 4.28mg g⁻¹ (100μM), 4.90mg g⁻¹ (500μM) in sodic soil.

In the present study the reduction in sugars in sugarcane leaves when grown under sodic soil may be due to losses in the photosynthetic activity with a drop in the net formation of carbohydrates in general (Trivedi *et al.*, 2004 and Gadallah, 1999). In cotton, foliar applications with Put increased total sugars under different levels of salinity compared to control plants (Ahmed *et al.*, 2013). Similar trend was observed in the present investigation. The increase in sugar in the present study by the application of PAs may have role act as osmoprotectants, regulating the cell osmotic status, protecting the

membranes, and contributing to the scavenging of free radicals in plant cells. (Leopold *et al.*, 1994).

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