The Pharma Innovation Journal 2022; 11(4): 1955-1964

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(4): 1955-1964

www.thepharmajournal.com
Received: 07-01-2022
Accepted: 19-02-2022

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Sugarcane plant growth promoting rhizo bacterial interaction for enhanced salinity tolerance

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DOI: https://doi.org/10.22271/tpi.2022.v11.i4ab.12242

Abstract
The present investigation was meted out on two promising sugarcane genotypes CoN-13072 and CoN-13073, were exposed to different NaCl levels to assess rhizobacterial interaction for salinity tolerance. From the shoot apex portion of two sugarcane genotypes tissue culture plantlets were developed. From that regenerated plantlets shifted to polyhouse and exposed to different NaCl levels to assess rhizobacterial interaction for salinity tolerance. Maximum shoot length (25.40 cm) was observed in genotype CoN-13073 under rhizobacterial inoculum at 2.0% NaCl concentration and maximum root length (12.40 cm) was observed at 0.0% and 1.5% NaCl concentration under rhizobacterial inoculum in genotype CoN-13072 and CoN-13073 respectively. Maximum shoot root ratio (2.60) and (2.40) was observed at 0.0% NaCl concentration with rhizobacterial inoculum within the rooting mixture of both the genotypes CoN-13073 and CoN-13072 respectively. High chlorophyll content index (5.48) and leaf area (41.20 cm^2/plant) was observed in genotype CoN-13073 at 0.0% NaCl concentration under rhizobacterial inoculum. In genotypes CoN-13073 and CoN-13072, with a rise in NaCl concentration upto 2.0% and 1.5% respectively, increase in shoot length, leaf area and chlorophyll content index was observed as a result of increase in ACC deaminase activity. ACC deaminase activity and ethylene content within the plant body are negatively correlated. Increase in ACC deaminase activity results decrease in ethylene synthesis in plant body to avoid stress. Among the 2 genotypes, CoN-13073 found to be more salt tolerant at higher NaCl levels with the exhibition of low osmotic conductance, maximum leaf area with high chlorophyll content index.

Keywords: Sugarcane, salinity, rhizo bacterial, NaCl, interaction

Introduction
The word sugarcane is springs from the Sanskrit word "*sharkara*" and it indicates its Indian origin (Sreenivasan *et al*. 1987) [20]. It's most vital crop and an industrial crop of the tropical and subtropical region of the globe thanks to its high trade value. Sugarcane is an important cash crop of South Gujarat region. Gujarat has 1600 km costal area which is largest area among all states in India, so some area of sugarcane cultivation littered with salt accumulated through sea water in addition as improper irrigation practices and sugarcane - paddy crop rotation is extremely common within the state which ends up in excessive use of water causing soil salinity. This situation decreased the production as well as the area of sugarcane cultivation in South Gujarat region. It includes 3 cultivated species like *S. officinarum*, *S. barberi*, and *S. sinense* (Zhang *et al*. 2012; Panje and Babu 1960) [22, 13]. The sugar industry is that the second largest agro-based industry in India. Sugarcane plays a serious role within the economy of sugarcane growing areas and hence improving sugarcane production will really helps in economic prosperity of the farmers and other associated industries with sugarcane cultivation. Because it grows under varied agro-climatic conditions, hence it faces various biotic and abiotic stresses that affects the productivity significantly. Salinity is one among the key abiotic stress which greatly affects the sugarcane productivity and recovery. The soils with electrical conductivity (EC) less than 4 dsm^{-1} are generally considered as salt-free, whereas soil with EC range between 4-8 dsm^{-1} are generally considered as moderately saline and soils with EC more than 8 dsm^{-1} are considered as strongly saline soil. Sugarcane could be a typical glycophyte (salt sensitive plants) and hence exhibits stunted growth or no growth under salinity, with its yield falling to 50% or less than its true potential (Subbarao and Shaw 1985). Salt stress affects several aspects of plant physiology by its osmotic and ionic components (Munns and Tester 2008) [12].
Microorganisms related to sugarcane play an important role in maintaining soil fertility and plant health. Many of those mutualistic organisms can act as biofertilizers, increasing the efficiency of nutrient absorption by the plant and producing substances that promote growth

and additionally to increasing tolerance to abiotic and biotic stresses by promoting biological control. In general, microorganisms involved in optimizing plant growth are denominated plant growth-promoting rhizobacteria (PGPR) (Rodrigues *et al*. 2016) [17]. Salinity could be a severe problem for temperate and tropical agriculture system, affecting 20% of world agriculture land. These PGPR tolerate wide range of salt stress and enable plants to face up to salinity by hydraulic conductance, osmotic accumulation, sequestering toxic Na^+ ions, maintaining the osmotic conductance and photosynthetic activities (Barashi *et al*., 2006) [2]. PGPR from stressed environment exhibit 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity which reduces the extent of endogenous ethylene, mitigating the deleterious effects of stress on overall plant growth. The plants inoculated with PGPR having ACC deaminase are relatively more tolerant to salt stress (Muhammad *et al*. 2007) [11]. So, present investigation was applied to screen two promising sugarcane genotypes CoN 13072 and CoN 13073 for salinity tolerance under endophytic bacterial environment and different salinity regimes at sugarcane tissue culture laboratories, Main Sugarcane Research Station, Navsari Agricultural University, Navsari. Various morphological and physiological parameters of plantlets at different salinity levels was studied.

Materials and Methods

Preparation of explants

The commercial cultivars of sugarcane CoN 13072 and CoN 13073 grown in Gujarat were used as the source of explants in this experiment. The explants were obtained from Main Sugarcane Research Station, Navsari Agricultural University, Navsari. The shoot apex of sugarcane were used as explants. Disease - free, genetically true-to-type and actively growing cane tops were selected from 5 to 7 months old sugarcane crop. Cane tops with the growing apices were cut approximately 10 cm long and washed thoroughly in running tap water for 30 minutes. Outer sheaths of cane tops were removed by wiping the sheath with rectified spirit. The shoots were then washed with soapy water (2 drops of Labonin into 250 ml of water) for about 5 to 6 minutes in a sterile 1-litre conical flask, followed by cleaning the materials with distilled water. The shoots were rinsed in 5 per cent sodium hypochlorite for 10 minutes. Then shoots were thoroughly rinsed in 70 per cent ethanol for 30 seconds followed by sterilizing double distilled water for 4-5 times till ethanol was completely washed out from the surface of the material. Surface sterilization was performed using 0.1 per cent mercuric chloride solution. Shoots were shaken vigorously for 5 minutes. Then the container was taken to the laminar clean air station. They were rinsed 3 to 4 times with sterile double distilled water to remove all traces of chemicals. The isolation of shoot apex of 1 to 1.5 cm was done by carefully removing the 2-3 outer whorls of the developing leaves with the help of a sterile sharp blade and inoculated on autoclaved MS medium. Good quality plantlets generated from the explants was selected for further experimentation to impose different levels of NaCl concentration and to check the response to salinity tolerance. The plantlets regenerated under various levels of NaCl concentration in the MS nutrient medium were shifted to polybags comprised of rooting mixture with rhizobacterial culture and different NaCl concentrations in polyhouse for hardening.

Isolation and characterization of PGPR

Soil samples were collected from NAU campus by considering diverse past cropping pattern. All the collected samples were characterized for different physical, chemical and biological parameters. Isolation of PGPR from collected soil samples was performed as per standard procedure given by Chakraborty *et al*. (2011) [3]. Briefly, 1 g soil was mixed with 25 ml of sterile distilled water followed by serial dilution and suspension was inoculated on nutrient agar (NA) plates. Well isolated colonies, with distinct morphology were transferred on fresh plate by four sector streaking method. Pure culture of isolate was transferred on the nutrient agar slant and was preserved at 4 °C temperature. Glycerol stocks were maintained for long term preservation of each isolates. Potent isolates were characterized in terms of colony characteristics (size, shape, elevation, margin, texture, pigmentation, opacity *etc*.) and morphological characteristics (cell shape, arrangement, Gram's reaction *etc*.). PGPR isolates were characterized and identified by 16S r-RNA sequencing (Molecular) method. Different isolated bacteria were screened for *in vitro* efficacy of plant growth promotion on the basis of Shoot length, root length, shoot root ratio, leaf area, chlorophyll content index, ACC deaminase activity, ethylene content and osmotic conductance as per standard procedures given by Chakraborty *et al*. (2011) [3].

The plantlets regenerated under various levels of NaCl concentration in the MS nutrient medium were shifted to polybags comprised of rooting mixture with rhizobacterial culture and different NaCl concentrations in polyhouse for hardening.

Observation recorded during experiment

The plantlets regenerated under various levels of NaCl concentration in the MS nutrient medium were shifted to polybags comprised of rooting mixture with rhizobacterial culture and different NaCl concentrations in polyhouse for hardening. Following observations were recorded during hardening. Five plantlets were randomly selected from each treatment on 55 days after planting and the length of the shoot was measured from the collar region to the tip of a topmost leaf. From the randomly selected plantlets the length of the root was measured from the collar region down to the tip of the longest root. The average value of shoot length and root length for each treatment was computed and recorded. Shoot root ratio was estimated by dry weight basis (Shomeili *et al*. 2011) [19]. The leaves from plants selected from each treatment were used for the estimation of leaf area after 60 days of planting. Leaf area was measured by leaf area meter (Model LI3000) and expressed as cm^2. The chlorophyll content index was recorded with the help of chlorophyll content meter (CCM - 200 Plus). It measures the absorbance of both wavelengths and calculates a Chlorophyll Content Index (CCI) value that is proportional to the amount of chlorophyll in the sample of each treatment. Parameters that symbolize the plant growth due to ACC deaminase activity were used to measure ACC deaminase activity. Following parameters were used shoot length, root length, leaf area and chlorophyll content index for ACC deaminase activity (Arshadullah *et al*. 2017) [1]. ACC deaminase activity and ethylene content in the plant body are negatively correlated. Increase in ACC deaminase activity results decrease in ethylene synthesis in plant body to avoid stress (Lamizadeh *et al*. 2016) [9]. Parameters like leaf area and chlorophyll content index were

used to measure osmotic conductance. Presence of rhizobacterial inoculum lower downs the osmotic conductance and avoids the rapid and severe reduction in leaf area and chlorophyll content index as salinity increases. Higher the leaf area and chlorophyll content index results in lower osmotic conductance and *vice versa* (Errabi *et al*. 2017) [5].

Statistical analysis

The data generated from the experiment were subjected to statistical analysis in Completely Randomized Design with factorial concept (FCRD) technique as suggested by Panse and Sukhatme (1985) [14].

Results and Discussion

The plantlets regenerated under various levels of NaCl concentration in the MS nutrient medium were shifted to polybags comprised of rooting mixture with rhizobacterial culture and different NaCl concentrations to study the effect and interaction of rhizobacterial culture under saline environment on plant growth.

Shoot length (cm)

In genotype CoN-13073, maximum shoot length (25.40 cm) was observed in treatment W_2T_4 (With rhizobacteria + 2.0% NaCl) followed by treatment W_2T_1 (With rhizobacteria + 0.0% NaCl) registered (24.60 cm) shoot length. Whereas minimum shoot length (9.40 cm) was observed in treatment W_1T_5 (Without rhizobacteria + 2.5% NaCl).

In genotype CoN-13072, maximum shoot length (19.40 cm) was observed in treatment W_2T_1 (With rhizobacteria + 0.0% NaCl) followed by treatment W_2T_3 (With rhizobacteria + 1.5% NaCl) registered (18.40 cm) shoot length. Whereas minimum shoot length (8.0 cm) was observed in treatment W_1T_5 (Without rhizobacteria + 2.5% NaCl). (Plate No. 1)

Overall comparision indicated that maximum shoot length (25.40 cm) was observed in genotype CoN-13073 under treatment level W_2T_4 (With rhizobacteria + 2.0% NaCl). There was little difference observed in shoot length within the treatment combination of rhizobacteria culture in genotype CoN-13073 (Table 1). Much difference in shoot length was observed in higher and lower levels of NaCl with and without rhizobacteria environment irrespective of genotypic differences. Lowest shoot length (8.0 cm) was observed in genotype CoN-13072 under treatment level W_1T_5 (Without rhizobacteria + 2.5% NaCl). In comparision with untreated culture, 50% treatments depicted higher average shoot length in both the genotypes. Generally, higher NaCl concentrations may induce reduced shoot length but incorporation of rhizobacterial culture in the rooting mixture increase the shoot length in both the genotypes. Similar results were obtained by Edkie *et al*. (2014) [4]; Kang *et al*. (2019) [7]; Priyanka and Amaresh (2018) [16].

Table 1: Effect of different NaCl concentrations with rhizobacterial interaction on shoot length (cm) of two sugarcane genotypes (after 55 days)

T (Concentration of NaCl)	G_1 (CoN-13073)			G_2 (CoN-13072)			Mean T	Mean W_1	Mean W_2
	W_1 (without rhizo-bacteria)	W_2 (with rhizo- bacteria)	Mean G_1	W_1 (without rhizo-bacteria)	W_2 (with rhizo-bacteria)	Mean G_2			
T_1 (NaCl-0.0%)	18.40	24.60	21.50	16.40	19.40	17.90	19.70	17.40	22.00
T_2 (NaCl-1.0%)	18.80	24.00	21.40	14.60	17.20	15.90	18.65	16.70	20.60
T_3 (NaCl-1.5%)	12.40	22.20	17.30	12.40	18.40	15.40	16.35	12.40	20.30
T_4 (NaCl-2.0%)	11.20	25.40	18.30	10.20	16.20	13.20	15.75	10.70	20.80
T_5 (NaCl-2.5%)	09.40	18.60	14.00	08.00	16.70	12.35	13.18	08.70	17.65
T_6 (NaCl-3.0%)	10.60	18.40	14.50	08.40	14.60	11.50	13.00	09.50	16.50
Mean GW	13.47	22.20	17.83	11.67	17.08	14.38	-	12.57	19.64
Effect	G	T	W	GT	TW	GW	GTW	CV %	
S.Em.+	0.05	0.08	0.05	0.11	0.11	0.06	0.16	1.71	
C.D. @ 5%	0.13	0.23	0.13	0.32	0.32	0.18	0.45		

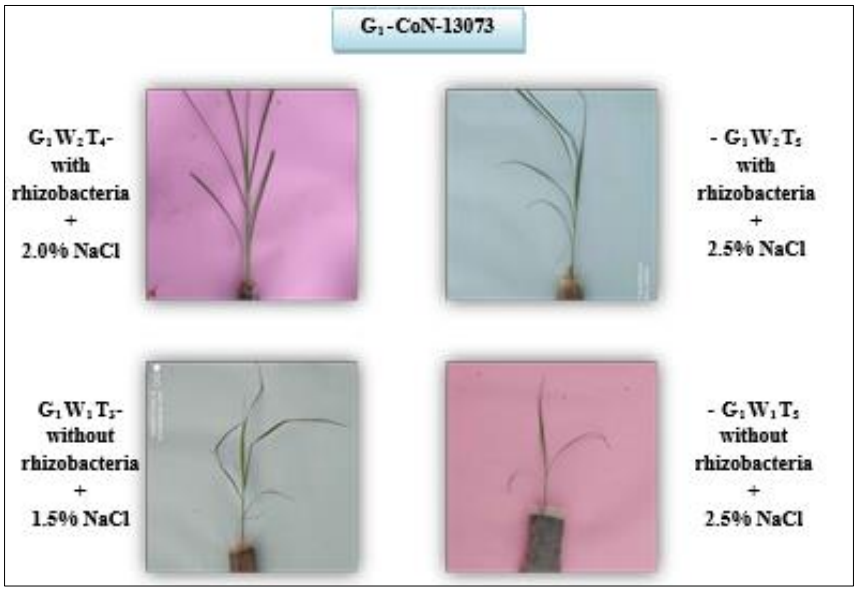

Plate 1: Effect of different NaCl concentrations with rhizobacterial interaction on shoot length (cm) of two sugarcane genotypes (after 55 days)

Root length (cm)

In genotype CoN-13073 plantlets regenerated in rooting mixture with rhizobacteria registered maximum root length (12.40 cm) in treatment W_2T_3 (With rhizobacteria + 1.5% NaCl) followed by treatment W_2T_1 (With rhizobacteria + 0.0% NaCl) registered (12.20 cm) root length. Whereas minimum root length (6.0 cm) was observed in treatment W_1T_6 (Without rhizobacteria + 3.0% NaCl).

In genotype CoN-13072, maximum root length (12.40 cm) was observed in treatment W_2T_1 (With rhizobacteria + 0.0% NaCl) followed by treatment W_1T_1 (Without rhizobacteria + 0.0% NaCl) registered (10.60 cm) root length. Whereas minimum root length (7.0 cm) was observed in treatment W_1T_6 (Without rhizobacteria + 3.0% NaCl). (Plate No. 2)

In overall comparision maximum root length (12.40 cm) was observed in genotype CoN-13073 under treatment level W_2T_3 (With rhizobacteria + 1.5% NaCl) and in genotype CoN-13072 under treatment level W_2T_1 (With rhizobacteria + 0.0% NaCl). Minimum root length (6.0 cm) was observed in genotype CoN-13073 under treatment level W_1T_6 (Without rhizobacteria + 3.0% NaCl) (Table 2). Plantlets derived from, treatment with rhizobacteria culture in the rooting mixture showed average root length of (10.40 cm) in CoN-13073 and (10.0 cm) in CoN-13072. Whereas rooting mixture without rhizobacteria culture showed average root length 8.0 cm in CoN-13073 and 8.20 cm in CoN-13072. Root length was influenced positively by the incorporation of rhizobacteria even at higher NaCl levels. Significant difference in root length was observed among the cultures with and without rhizobacteria in both the genotypes. Inhibitory effect was observed due to higher NaCl concentrations. Similar results were observed by Edkie *et al*. (2014) [4]; Saxena *et al*. (2010) [18]; Priyanka and Amaresh (2018) [16].

Table 2: Effect of different NaCl concentrations with rhizobacterial interaction on root length (cm) of two sugarcane genotypes (after 55 days)

T (Concentration of NaCl)	G1 (CoN-13073)			G2 (CoN-13072)			Mean T	Mean W1	Mean W2
	W1 (without rhizo-bacteria)	W2 (with rhizo- bacteria)	Mean G1	W1 (without rhizo-bacteria)	W2 (with rhizo-bacteria)	Mean G2			
T1 (NaCl-0.0%)	10.40	12.20	11.30	10.60	12.40	11.50	11.40	10.50	12.30
T2 (NaCl-1.0%)	10.00	11.60	10.80	09.40	10.00	09.70	10.25	09.70	10.80
T3 (NaCl-1.5%)	08.40	12.40	10.40	10.00	10.40	10.20	10.30	09.20	11.40
T4 (NaCl-2.0%)	08.00	11.40	09.70	08.20	08.40	08.30	09.00	08.10	09.90
T5 (NaCl-2.5%)	06.20	08.40	07.30	07.20	10.20	08.70	08.00	06.70	09.30
T6 (NaCl-3.0%)	06.00	06.80	06.40	07.00	08.70	07.85	07.13	06.50	07.75
Mean GW	08.17	10.47	09.32	08.73	10.02	09.38	-	08.45	10.24
Effect	G	T	W	GT	TW	WG	GTW	CV %	
S.Em.+	0.04	0.06	0.04	0.09	0.09	0.05	0.13	2.36	
C.D. @ 5%	NS	0.18	0.10	0.26	0.26	0.15	0.36		

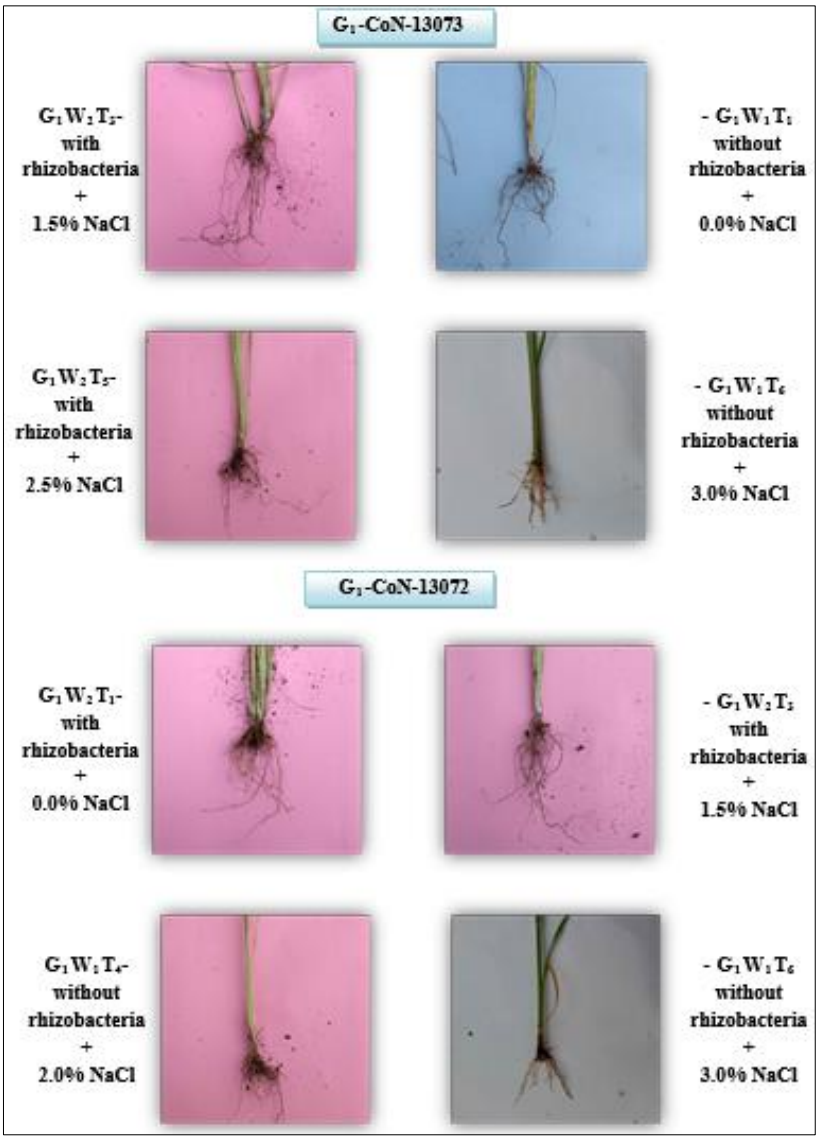

Plate 2: Effect of different NaCl concentrations with rhizobacterial interaction on root length (cm) of two sugarcane genotypes (after 55 days)

Shoot root ratio

After full establishment of plantlets, shoot root ratio was recorded. In genotype CoN-13073, maximum shoot root ratio (2.60) was observed in treatment W_2T_1 (With rhizobacteria + 0.0% NaCl) followed by shoot root ratio (2.40) under treatment level W_2T_3 and W_2T_4 (With rhizobacteria + 1.5% and 2.0% NaCl concentration respectively). Whereas minimum shoot root ratio (1.30) was observed in treatment W_1T_6 (Without rhizobacteria + 3.0% NaCl).

In genotype CoN-13072, maximum shoot root ratio (2.40) was observed in treatment W_2T_1 (With rhizobacteria + 0.0% NaCl). Whereas minimum shoot root ratio (1.40) was observed in treatment W_1T_6 (Without rhizobacteria + 3.0% NaCl). (Table 3)

Among both the genotypes maximum shoot root ratio (2.60) was observed in treatment W_2T_1 (With rhizobacteria + 0.0% NaCl). Average shoot root ratio was found high in genotype CoN-13073 where rhizobacteria culture influenced positively towards high biomass yield irrespective of the NaCl concentration. Whereas plantlets regenerated without rhizobacteria culture exhibited poor shoot root ratio in both the genotypes. Higher concentrations of NaCl above 2.0% resulted into poor shoot root ratio in the plantlets without rhizobacteria. Incorporation of rhizobacterial culture in the rooting mixture provided favourable environment in rhizosphere for maximum uptake of available nutrients under high NaCl concentrations. Similar results were observed by Edkie *et al*. (2014) [4]; Priyanka and Amaresh (2018) [16].

Table 3: Effect of different NaCl concentrations with rhizobacterial interaction on shoot root ratio of two sugarcane genotypes

T (Concentration of NaCl)	G_1 (CoN-13073)			G_2 (CoN-13072)			Mean T	Mean W_1	Mean W_2
	W_1 (without rhizo-bacteria)	W_2 (with rhizo-bacteria)	Mean G_1	W_1 (without rhizo-bacteria)	W_2 (with rhizo-bacteria)	Mean G_2			
T_1 (NaCl-0.0%)	2.40	2.60	2.50	2.20	2.40	2.30	2.40	2.30	2.50
T_2 (NaCl-1.0%)	2.20	2.20	2.20	2.20	2.20	2.20	2.20	2.20	2.20
T_3 (NaCl-1.5%)	1.80	2.40	2.10	2.00	2.00	2.00	2.05	1.90	2.20
T_4 (NaCl-2.0%)	1.80	2.40	2.10	1.60	1.80	1.70	1.90	1.70	2.10

T_5 (NaCl-2.5%)	1.60	2.00	1.80	1.50	1.80	1.65	1.73	1.55	1.90
T_6 (NaCl-3.0%)	1.30	2.00	1.65	1.40	1.60	1.50	1.58	1.35	1.80
Mean GW	1.85	2.27	2.06	1.82	1.97	1.89	-	1.83	2.12
Effect	G	T	W	GT	TW	WG	GTW	CV %	
S.Em.+	0.01	0.02	0.01	0.03	0.03	0.01	0.04	3.19	
C.D. @ 5%	0.03	0.05	0.03	0.07	0.07	0.04	0.10		

Leaf area (cm^2/plant)

Plantlets developed from various treatments were considered for leaf area. In genotype CoN-13073, maximum leaf area (41.20 cm^2/plant) was observed in treatment W_2T_1 (With rhizobacteria + 0.0% NaCl) followed by treatment W_2T_3 (With rhizobacteria + 1.5% NaCl) registered (40.60 cm^2/plant) leaf area. Whereas minimum leaf area (31.20 cm^2/plant) was observed in treatment W_1T_6 (Without rhizobacteria + 3.0% NaCl).

In genotype CoN-13072, maximum leaf area (40.20 cm^2/plant) was observed in treatment W_1T_1 (Without rhizobacteria + 0.0% NaCl) followed by treatment W_1T_2 (Without rhizobacteria + 1.0% NaCl) registered (38.60 cm^2/plant) leaf area. Whereas minimum leaf area (28.00 cm^2/plant) was observed in treatment W_1T_6 (Without rhizobacteria + 3.0% NaCl). (Table 4)

The plantlets established at different salinity levels showed differences for leaf area. High leaf area (41.20 cm2/plant) was observed in treatment W_2T_1 (With rhizobacteria + 0.0% NaCl). Minimum leaf area was observed in the culture where 3.0% NaCl was incorporated without rhizobacteria in both the genotypes. Increase in leaf area was positively associated with shoot length, root length and chlorophyll content in both the genotypes. Rhizobacteria culture in the rooting mixture favoured growth during the early stage irrespective of salinity condition. These results are in agreement with Pirhadi *et al*. (2017) [15]; Saxena *et al*. (2010) [18].

Table 4: Effect of different NaCl concentrations with rhizobacterial interaction on leaf area (cm^2/plant) of two sugarcane genotypes

T (Concentration of NaCl)	G_1 (CoN-13073)			G_2 (CoN-13072)			Mean T	Mean W_1	Mean W_2
	W_1 (without rhizo-bacteria)	W_2 (with rhizo- bacteria)	Mean G_1	W_1 (without rhizo-bacteria)	W_2 (with rhizo-bacteria)	Mean G_2			
T_1 (NaCl-0.0%)	37.40	41.20	39.30	40.20	37.60	38.90	39.10	38.80	39.40
T_2 (NaCl-1.0%)	36.80	40.40	38.60	38.60	37.00	37.80	38.20	37.70	38.70
T_3 (NaCl-1.5%)	36.70	40.60	38.65	32.40	35.60	34.00	36.33	34.55	38.10
T_4 (NaCl-2.0%)	34.20	38.40	36.30	30.60	34.20	32.40	34.35	32.40	36.30
T_5 (NaCl-2.5%)	31.80	35.20	33.50	28.70	32.40	30.55	32.03	30.25	33.80
T_6 (NaCl-3.0%)	31.20	34.00	32.60	28.00	30.50	29.25	30.93	29.60	32.25
Mean GW	34.68	38.30	36.49	33.08	34.55	33.82	-	33.88	36.43
Effect	G	T	W	GT	TW	WG	GTW	CV %	
S.Em.+	0.05	0.09	0.05	0.13	0.13	0.07	0.18	0.89	
C.D. @ 5%	0.15	0.26	0.15	0.36	0.36	0.21	0.51		

Chlorophyll content index

Chlorophyll content index in shoots of plantlets developed under rhizobacterial environment decreased with increase in NaCl concentrations. In genotype CoN-13073, maximum chlorophyll content index (5.48) was observed in treatment W_2T_1 (Without rhizobacteria + 0.0% NaCl) followed by chlorophyll content index (5.36) under treatment level W_2T_2 and W_2T_5 (With rhizobacteria + 1.0% and 2.5 NaCl concentration respectively). Whereas minimum chlorophyll content index (3.54) was observed in treatment W_1T_6 (Without rhizobacteria + 3.0% NaCl).

In genotype CoN-13072, maximum chlorophyll content index (4.82) was observed in treatment W_2T_1 (Without rhizobacteria + 0.0% NaCl). Whereas minimum chlorophyll content index (3.0) was observed in treatment W_1T_6 (Without rhizobacteria + 3.0% NaCl). (Table 5)

The results showed that high chlorophyll content index was observed in plantlets regenerated under rhizobacteria, whereas poor chlorophyll content index was reported in plantlets regenerated without rhizobacteria in genotype CoN-13072. In both genotypes, at 0.0% NaCl concentration with rhizobacteria showed maximum chlorophyll content index. Increase in the levels of NaCl reduced chlorophyll content index. These results are in agreement with Shomeili *et al*. (2011) [19]; Lamizadeh *et al*. (2016) [9].

Table 5: Effect of different NaCl concentrations with rhizobacterial interaction on chlorophyll content index of two sugarcane genotypes

T (Concentration of NaCl)	G_1 (CoN-13073)			G_2 (CoN-13072)			Mean T	Mean W_1	Mean W_2
	W_1 (without rhizo-bacteria)	W_2 (with rhizo- bacteria)	Mean G_1	W_1 (without rhizo-bacteria)	W_2 (with rhizo-bacteria)	Mean G_2			
T_1 (NaCl-0.0%)	4.64	5.48	5.06	4.42	4.82	4.62	4.84	4.53	5.15
T_2 (NaCl-1.0%)	4.48	5.36	4.92	4.26	4.64	4.45	4.69	4.37	5.00
T_3 (NaCl-1.5%)	4.40	5.30	4.85	3.84	4.62	4.23	4.54	4.12	4.96
T_4 (NaCl-2.0%)	4.30	5.32	4.81	3.62	4.44	4.03	4.42	3.96	4.88
T_5 (NaCl-2.5%)	3.82	5.36	4.59	3.34	3.80	3.57	4.08	3.58	4.58
T_6 (NaCl-3.0%)	3.54	4.80	4.17	3.00	3.60	3.30	3.74	3.27	4.20
Mean GW	4.20	5.27	4.73	3.75	4.32	4.03	-	3.97	4.80

Effect	G	T	W	GT	TW	WG	GTW	CV %
S.Em.+	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.70
C.D. @ 5%	0.01	0.03	0.01	0.04	0.04	0.02	0.05	

ACC deaminase activity

Generally ACC deaminase producing bacteria assist plant growth and effectively protect to salinity stress. Rhizobacteria containing ACC deaminase phenomena, lower downs the plant ethylene levels during the adverse environmental stress conditions. Parameter that symbolize plant growth due to ACC deaminase activity are shoot length, root length, leaf area, chlorophyll content index and photosynthetic rate. Higher the leaf area and chlorophyll content index more is the photosynthesis and better the plant growth. Supplementation of rhizobacterial inoculum results in maximum increase in shoot length, root length and leaf area upto moderate levels of NaCl concentration.

In genotype CoN-13073, with an increase in NaCl concentration upto 2% increase in shoot length, leaf area and chlorophyll content index in treatment W_2T_1 to W_2T_4 under rhizobacterial inoculum was observed as a result of ACC deaminase activity. In other words reduction in shoot length, leaf area and chlorophyll content index was observed at higher NaCl concentration without rhizobacterial inoculum results in reduction of ACC deaminase activity.

In genotype CoN-13072, ACC deaminase activity increased due to rhizobacterial inoculum in treatments W_2T_1, W_2T_2 and W_2T_3 upto 1.5% NaCl concentration. At higher concentrations of NaCl above 2.5% reduction in ACC deaminase activity was observed. Overall, both the genotypes at genetic level responded to rhizobacterial inoculum to asses ACC deaminase activity considering growth parameters in the study. (Table 6)

ACC deaminase activity was found strongly in rhizobacterial inoculum treated culture in genotype CoN-13073. Whereas no active phenomena was observed in without rhizobacterial inoculum in both the genotypes. Both the genotypes responded significantly with incorporation of rhizobacterial culture under salinity stress and expression of growth parameter was clear in treated and untreated culture material. Similar findings were reported by Arshadullah *et al*. (2017) [1]; Lamizadeh *et al*. (2016) [9].

Table 6: Effect of different NaCl concentrations with rhizobacterial interaction of ACC deaminase activity and ethylene content on plant growth of two sugarcane genotypes

Sr. No.	Shoot length (cm)	Root length (cm)	Leaf area (cm^2/plant)	Chlorophyll content index	ACC deaminase activity	Ethylene content
$G_1W_1T_1$	18.40	10.40	37.40	4.64	-	+
$G_1W_1T_2$	18.80	10.00	36.80	4.48	-	+
$G_1W_1T_3$	12.40	08.40	36.70	4.40	-	+
$G_1W_1T_4$	11.20	08.00	34.20	4.30	-	+
$G_1W_1T_5$	09.40	06.20	31.80	3.82	-	+
$G_1W_1T_6$	10.60	06.00	31.20	3.54	-	+
$G_1W_2T_1$	24.60	12.20	41.20	5.48	+++	-
$G_1W_2T_2$	24.00	11.60	40.40	5.36	++	-
$G_1W_2T_3$	22.20	12.40	40.60	5.30	+++	-
$G_1W_2T_4$	25.40	11.40	38.40	5.32	++	-
$G_1W_2T_5$	18.60	08.40	35.20	5.36	-	+
$G_1W_2T_6$	18.40	06.80	34.00	4.80	-	+
$G_2W_1T_1$	16.40	10.60	40.20	4.42	-	+
$G_2W_1T_2$	14.60	09.40	38.60	4.26	-	+
$G_2W_1T_3$	12.40	10.00	32.40	3.84	-	+
$G_2W_1T_4$	10.20	08.20	30.60	3.62	-	+
$G_2W_1T_5$	08.00	07.20	28.70	3.34	-	+
$G_2W_1T_6$	08.40	07.00	28.00	3.00	-	+
$G_2W_2T_1$	19.40	12.40	37.60	4.82	++	-
$G_2W_2T_2$	17.20	10.00	37.00	4.64	++	-
$G_2W_2T_3$	18.40	10.40	35.60	4.62	+	-
$G_2W_2T_4$	16.20	08.40	34.20	4.44	-	+
$G_2W_2T_5$	16.70	10.20	32.40	3.80	-	+
$G_2W_2T_6$	14.60	08.70	30.50	3.60	-	+

Note: + Low active, ++ Moderate active, +++ Strong active, - No active

Ethylene content

ACC deaminase activity and ethylene content in the plant body are negatively correlated. Increase in ACC deaminase activity results decrease in ethylene synthesis in plant body to avoid stress. Growth parameters studied proven reduction in ethylene content in most of the rhizobacterial inoculum treatments in both the genotypes. Reduction in growth rate was observed in treatments T_1 to T_6 in both the genotypes where incorporation of NaCl concentrations exhibited stress environment in the rhizosphere where no incorporation of rhizobacterial inoculum in the culture. Whereas in treatments T_1 to T_6 in both the genotypes where incorporation of rhizobacterial inoculum in the culture was done showed vigorous growth rate for shoot length, root length, chlorophyll content index and leaf area. Rhizobacterial inoculum played significant role for stress tolerance with reduced levels of ethylene for most of the growth parameters.

Overall both the genotypes responded to stress environment and effect of rhizobacterial inoculum exhibited increased growth rate with the reduced levels of ethylene synthesis in biochemical pathway.

High ethylene synthesis in the absence of rhizobacteria

culture resulted poor shoot length, leaf area, and chlorophyll content index in both the genotypes. Similarly, high NaCl concentration above 2.0% exhibited high ethylene synthesis resulted in poor growth parameters (Table 6). Rhizobacteria inoculum treated plantlets exhibited significant growth under saline stress environment upto (2.0% NaCl). At genetic level, both the genotypes responded to salinity stress with regards to growth expression. Genotype CoN-13073 found to be more stress tolerant under the rhizobacterial inoculum incorporation. Similar results were observed by Mirza *et al*. (2001) [10]; Lamizadeh *et al*. (2016) [9].

Osmotic conductance

Stomatal conductance used as a one of the screening technique for osmotic stress tolerance. Basically stomatal response is due to the osmotic pressure as a result of osmotic conductance of the salts outside the roots and not the Na^+ and Cl^- taken up by the plant. Osmotic conductance initiates the signaling mechanism for stomatal conductance. Higher the sodium levels in the rhizosphere higher the stomatal conductance and results higher osmotic conductance. In the resistant genotypes presence of rhizobacterial inoculum lower downs the osmotic conductance and avoids the rapid and severe reduction in leaf area expansion and chlorophyll content index as salinity increases.

The results obtained in the present study shows that in genotype CoN-13073, increase in salinity levels above 2.0% reduction in the leaf area and chlorophyll content index was observed in treatments W_1T_4, W_1T_5 and W_1T_6 without rhizobacteria and treatment W_2T_6 with rhizobacteria. In genotype CoN-13072, also increase in salinity levels above 2.0% reduction in the leaf area and chlorophyll content index was observed in treatments W_1T_4, W_1T_5 and W_1T_6 without rhizobacteria and treatments W_2T_5 and W_2T_6 with rhizobacteria. That indicate the increased osmotic adjustment at above 2.0% NaCl was observed in both the genotypes. Among the two genotypes CoN-13073 found to be more tolerance at higher NaCl levels with exhibition of low osmotic conductance and maximum leaf area with high chlorophyll content index. The treatments with rhizobacterial inoculum in both the genotypes suppressed the osmotic conductance upto 2.0% NaCl concentration. (Table 7)

Genotype CoN-13073 exhibited low osmotic conductance at higher NaCl concentration of 2.5% under rhizobacterial inoculum. Whereas untreated culture exhibited high osmotic conductance with reduced leaf area and low chlorophyll content index. Among both the genotypes, CoN-13073 performed well under saline stress condition with reduced osmotic conductance by avoiding the absorption of high amount of Na^+ and Cl^- ions from rhizosphere, at the same time prevents the leaf area reduction and chlorophyll content index for better survival under high saline conditions. Similar results were reported by Errabi *et al*. (2017) [5]; Gandonou *et al*. (2006) [6]; Karpe *et al*. (2012) [8].

Table 7: Effect of different NaCl concentrations with rhizobacterial interaction of osmotic conductance on plant growth of two sugarcane genotypes

Sr. No.	Leaf area (cm^2/plant)	Chlorophyll content index	Osmotic conductance
$G_1W_1T_1$	37.40	4.64	Low
$G_1W_1T_2$	36.80	4.48	Low
$G_1W_1T_3$	36.70	4.40	Low
$G_1W_1T_4$	34.20	4.30	High
$G_1W_1T_5$	31.80	3.82	High
$G_1W_1T_6$	31.20	3.54	High
$G_1W_2T_1$	41.20	5.48	Low
$G_1W_2T_2$	40.40	5.36	Low
$G_1W_2T_3$	40.60	5.30	Low
$G_1W_2T_4$	38.40	5.32	Low
$G_1W_2T_5$	35.20	5.36	Low
$G_1W_2T_6$	34.00	4.80	High
$G_2W_1T_1$	40.20	4.42	Low
$G_2W_1T_2$	38.60	4.26	Low
$G_2W_1T_3$	32.40	3.84	Low
$G_2W_1T_4$	30.60	3.62	High
$G_2W_1T_5$	28.70	3.34	High
$G_2W_1T_6$	28.00	3.00	High
$G_2W_2T_1$	37.60	4.82	Low
$G_2W_2T_2$	37.00	4.64	Low
$G_2W_2T_3$	35.60	4.62	Low
$G_2W_2T_4$	34.20	4.44	Low
$G_2W_2T_5$	32.40	3.80	High
$G_2W_2T_6$	30.50	3.60	High

Note: Low osmotic conductance: High salinity tolerance High osmotic conductance:- Poor salinity tolerance

Table 8: Mean values of the characters of two sugarcane genotypes

Sr. No.	Shoot length (cm)	Root length (cm)	Shoot root ratio	Leaf area (cm^2/plant)	Chlorophyll content index
$G_1W_1T_1$	18.40	10.40	2.40	37.40	4.64
$G_1W_1T_2$	18.80	10.00	2.20	36.80	4.48
$G_1W_1T_3$	12.40	8.40	1.80	36.70	4.40
$G_1W_1T_4$	11.20	8.00	1.80	34.20	4.30
$G_1W_1T_5$	9.40	6.20	1.60	31.80	3.82
$G_1W_1T_6$	10.60	6.00	1.30	31.20	3.54
$G_1W_2T_1$	24.60	12.20	2.60	41.20	5.48
$G_1W_2T_2$	24.00	11.60	2.20	40.40	5.36
$G_1W_2T_3$	22.20	12.40	2.40	40.60	5.30
$G_1W_2T_4$	25.40	11.40	2.40	38.40	5.32
$G_1W_2T_5$	18.60	8.40	2.00	35.20	5.36
$G_1W_2T_6$	18.40	6.80	2.00	34.00	4.80
$G_2W_1T_1$	16.40	10.60	2.20	40.20	4.42
$G_2W_1T_2$	14.60	9.40	2.20	38.60	4.26
$G_2W_1T_3$	12.40	10.00	2.00	32.40	3.84
$G_2W_1T_4$	10.20	8.20	1.60	30.60	3.62
$G_2W_1T_5$	8.00	7.20	1.50	28.70	3.34
$G_2W_1T_6$	8.40	7.00	1.40	28.00	3.00

$G_2W_2T_1$	19.40	12.40	2.40	37.60	4.82
$G_2W_2T_2$	17.20	10.00	2.20	37.00	4.64
$G_2W_2T_3$	18.40	10.40	2.00	35.60	4.62
$G_2W_2T_4$	16.20	8.40	1.80	34.20	4.44
$G_2W_2T_5$	16.70	10.20	1.80	32.40	3.80
$G_2W_2T_6$	14.60	8.70	1.60	30.50	3.60

16' s r-DNA sequence

***Myroides profundi* (PGPR-1)**
TTGATGCCTCGGCTCAGCAAGGATACGTCGCTAAGGGTGTCCGCGAAGAGTGTGCGTGTGCCTCACGAGTATGCAACCTACCTT
ATACAGGGGAATAGCCCGAAGAAATTCGGATTAATGCTCCATGGTTTATCGATATGGCATCGTATTGATAATAAAGATTTATCG
GTATAAGATGGGCATGCGTATCATTAGCTAGTTGGTGTGGTAACGGCATACCAAGGCAACGATGATTAGGGGTCCTGAGAGGG
AGATCCCCCACACTGGTACTGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATTGGTCAATGGAGGCAACTC
TGAACCAGCCATGCCGCGTGCAGGATGACGGTCCTATGGATTGTAAACTGCTTTTGTACAGGAAGAAACCTCCCTACGAGTAGG
GACTTGACGGTACTGTAAGAATAAGGATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATCCGAGCGTTATCCGG
AATTATTGGGTTTAAAGGGTTCGTAGGCGGCTTTGTAAGTCAGTGTGGAAATTTCCTAGCTTAACTAGGACACTGCCATTGATA
CTGCAAAGCTTGAATA

Conclusion

From the study it is concluded that the salt tolerance and symbiotic association are the characteristics of some microbes. Salt tolerant microbes can survive in osmotic and ionic stresses. Various rhizobacteria which were previously identified in salt affected areas are also known to mitigate various biotic and abiotic stresses in plants. Incorporation of rhizobacterial inoculum in the rooting mixture enhance the growth of plant upto moderate levels of NaCl in both the genotypes. Among the two genotypes, CoN-13073 found to be more salt tolerant under rhizobacterial environment at higher NaCl levels with the exhibition of low osmotic conductance and maximum leaf area with high chlorophyll content index. There is immense need to study varietal interaction with existing salinity tolerant rhizobacteria.

Acknowledgments

This study was supported by the Main Sugarcane Research Station and Department of Genetics and Plant Breeding, N. M. College of Agriculture, Navsari Agricultural University, Navsari. The motivating guidance of my major advisor Dr. S. C. Mali, Associate Research Scientist at Main Sugarcane Research Station and Dr. Jagdish udutha, who was a constant supporter and gave his useful suggestions, invaluable guidance, constructive counsel and unreserved help that served as a beckoning light throughout the period of course study as well as research work.

Conflict of Interest

The authors declares that there is no conflict of interest.

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