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Bacillus subtilis Isolated from the Sugarcane Root Rhizosphere: A Potential Bioinoculum to Alleviate Salinity Stress in Sugarcane Cultivation

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ABSTRACT

Symbiotic association of rhizospherial organisms helps plants for better adaptability and acquisition of nutrients required for sustainable growth and productivity. *Bacillus subtilis* isolated from the root rhizosphere of salinity tolerant sugarcane variety, CoM 0265 was evaluated as PGPR using commercially grown cultivar of sugarcane, Co 86032 exposed to salinity stress under greenhouse conditions. Morphological parameters viz., root- shoot length, leaf number, chlorophyll, cations like Mn, Cu, Fe and K showed significant increase with the *Bacillus* inoculum. MDA, proline, reducing sugar, protein, ascorbate peroxidase and catalase content, minerals like Na and Zn showed an increase with saline stress, but supplementing *Bacillus* inoculum caused significant reduction in these components and protected plants from salinity stress. The results of the study put together suggest that this *Bacillus* isolate has potential to be used as bioinoculum in sugarcane cultivation and improve productivity.

Key words: Antioxidants, *Bacillus subtilis*, bioinoculum, salinity, sugarcane, nutrients

INTRODUCTION

Salinity is a major abiotic environmental factor which affects plant growth and productivity. Approximately 23% of the cultivated lands are considered as saline and 37% are sodic. Salinity as well as water logging seriously contributes to land deterioration and about 2% of the arable land is becoming unproductive each year, posing a serious threat to agriculture in South and Southeast Asian countries (DasGupta and Shaw, 2013; Gilman *et al.*, 2008; Polidoro *et al.*, 2010). Salinity in general, inhibits plant growth by affecting water absorption and physiological processes especially associated with nitrogen and CO_2 assimilation. Saline conditions drastically change the root environment, osmotic potential of soil solution and normal equilibrium of the dissolved ions. Availability of micronutrients to crop plants depends mainly on the pH of the soil solution as well as the surface nature of the binding sites on organic and inorganic particles (Achakzai *et al.*, 2010; Violante *et al.*, 2010; Kumar and Babel, 2011).

Plants under salinity stress accumulate osmoprotectants, like proline, glucose, glycine betaine, which play a crucial role in membrane potential and osmoregulation. ROS (Reactive Oxygen

Species) causes oxidative damage to biomolecules and eventually leads to cell death. Antioxidant enzymes such as superoxide dismutase, catalase and levels of antioxidants are alleviated in response to stress, in turn protect cells from stress and oxidative damage.

Application of PGPR (Plant Growth-Promoting Rhizobacteria) like *Bacillus*, *Pseudomonas*, *Serratia* and *Rhizobium* spp. have shown to be beneficial to crops and are reported to enhance growth and productivity. They are reported to improve germination rate, tolerance to drought, improve shoot and root growth and yield under salinity stress. Further they are found to secrete antibacterial and bio protective compounds which are effective against plant pathogens and pests, facilitate enhanced uptake of nitrogen and solubilisation of recalcitrant phosphorus and its assimilation and production of siderophores to facilitate the absorption of minerals (Herman *et al*., 2008; Ashrafuzzaman *et al*., 2009; Richardson *et al*., 2009). The present study reports on the efficacy of using *Bacillus* spp. isolated from the sugarcane root rhizosphere as bio-inoculum to protect sugarcane cultivar from salinity stress. Salinity is a measure of concentration of soluble salts in the soil. Sugarcane is sensitive to salinity stress (EC<2 dS m^{-1}) and high salt levels adversely affect plant growth. Higher EC inhibit the roots from absorbing mineral nutrients leading to nutrient deficiency (Sharp and Davies, 2009; Macedo and Jan, 2008). Green house experiments were carried out to examine the effectiveness of *Bacillus* spp. as bio-inoculum to protect sugarcane cultivar, CoC 86032, from salinity stress and changes in the morphometric and physiological parameters. The results of the experiment suggest that *Bacillus* show beneficial effect on plant growth under normal and saline conditions imposed in this study.

MATERIALS AND METHODS

Isolation of *Bacillus* spp.: Soil sample from the root rhizosphere of sugarcane variety CoM 0265 (saline tolerant) was collected from Sugarcane Research Station, Padegoan, Maharashtra, India. Soil suspension (1:100 w/v) in sterile water was serially diluted and plated on LB agar containing 1-5% NaCl. *Bacillus* spp. that grew on LB with 5% NaCl was isolated and was identified as *B. subtilis*. The culture was maintained on Luria broth containing 5% NaCl for further studies.

Experimental design: The experiments were carried out in 5 kg plastic bags under green-house conditions (16/8 h photoperiod; 70 μE m^2 sec^{-1}; 26°C). Seedlings of sugarcane (*Saccharum officinarum* L.) cultivar cv. Co 86032 was raised in pots with soil mix (soil: vermiculite: organic fertilizer, 3:2:1 w/w) and initially equilibrated with NaCl to adjust the salinity levels to 1 and 2% (4.5 and 7.5 mohs), respectively. *Bacillus* culture in LB with 5% NaCl (1.0 OD at 600 nm, 10^8 cell mL^{-1}) was used as inoculum (25 mL 2.0 kg^{-1} of soil mix). Buds of plants dipped in the culture for 30 min were sown in these pots. Twenty pots (20 replicates, one plant per pot) of each

Table 1: Treatment details of the pot experiments carried out for plant growth and physiological studies in sugarcane cultivar CoC 86032

Treatment No.	Treatment for pot experiment	Treatment No.	Treatment for pot experiment
1	Unautoclaved soil	6	Autoclaved soil
2	Unautoclaved soil+*Bacillus*	7	Autoclaved soil+*Bacillus*
3	Unautoclaved soil+1% NaCl	8	Autoclaved soil+1% NaCl
4	Unautoclaved soil+1% NaCl+*Bacillus*	9	Autoclaved soil+1% NaCl+*Bacillus*
5	Unautoclaved soil+2% NaCl+*Bacillus*	10	Autoclaved soil+2% NaCl+*Bacillus*

Table 2: Effect of *Bacillus* on cell count, leaf chlorophyll, MDA, proline, sugar, protein, cations and antioxidant activity under salinity stress

						Antioxidants		Nutrient ions			
	Tot Chl	MDA	Proline	RS	Protein	Catalase	Ascorbate	Fe	Zn	Cu	Mn
Treatment*	mg g^{-1} FW	----------------μg g^{-1} FW----------------				(Units mg^{-1} protein)		----------ppm in LDW----------			
Unautoclaved soil	81.90	0.32	60.97	71.35	319.90	5.79	2.36	621.00	40.20	27.70	52.43
Unautoclaved soil+*Bacillus*	123.95	0.20	53.11	47.35	117.19	4.24	1.87	772.60	26.70	37.70	72.33
Unautoclaved soil+1% NaCl	42.46	0.56	107.00	197.33	757.37	9.85	52.77	260.30	46.00	28.89	38.76
Unautoclaved soil+1% NaCl+*Bacillus*	107.96	0.43	82.63	149.82	553.36	7.82	36.94	347.00	27.00	33.39	68.52
Unautoclaved soil+2% NaCl+*Bacillus*	39.17	0.78	190.09	328.11	864.31	13.61	54.14	163.50	69.00	17.33	25.06
Autoclaved Soil	92.69	0.40[a]	74.97	91.81[a]	429.94	7.26	19.22	450.70	47.70	35.30	57.50
Autoclaved soil+*Bacillus*	100.92	0.37[a]	61.39	83.62[a]	361.33	6.58	16.91	519.50	36.00	38.68	105.11
Autoclaved soil+1% NaCl	67.66	0.65	185.65	193.06[a]	869.07	14.24	62.35	186.50	50.00	20.83	38.90
Autoclaved soil+1% NaCl+*Bacillus*	86.40	0.70	136.62	177.69[a]	640.01	8.46	39.11	288.50	30.00	26.26	68.76
Autoclaved soil+2% NaCl+*Bacillus*	37.58	1.16	250.87	200.53	998.91	15.11	81.03	130.50	88.00	13.40	20.91

a: Value not significant at 0.1 and 0.05% significance level as determined by Student's T-test, Remaining values are significant at 0.05%, 0.1 and 0.2 % significance level, *Sample size for the treatments is 20, data is mean and the standard error (SE) calculated in each case is at 95% confidence level, Tot Chl: Total chlorophyll, MDA: Malondialdehyde content (oxidative damage to lipids), RS: Reducing sugar, FW: Fresh weight, LDW: Leaf dry weight

treatment were maintained by watering daily to maintain the moisture content (Table 1). Precautions were taken to maintain the salinity level by monitoring the EC. After 75 days, leaf samples were collected for assay of physico-chemical parameters (Table 2).

Morphological parameters and chemical analysis: Proline (Bates *et al*., 1973), Chlorophyll (Witham *et al*., 1971), catalase (Chance and Maehly, 1955), Ascorbate peroxidase (Nakano and Asada, 1981) reducing sugars (Miller, 1972), soluble Protein (Bradford, 1976) and MDA (Stanley *et al*., 1975) content were determined following standard assay methods. The morphological parameters like root and shoot length, leaf number as well as water retention ability (Jia *et al*., 2008) were recorded. For the estimation of the cations (Na, Mn, K, Fe, Zn and Cu), 1.0 g of leaf samples were digested with acid-mixture (HNO_3:$HClO_4$, 1:1 v/v) and the volume was made up to 50 mL. The cation concentration was determined using AAS using suitable standards.

RESULTS AND DISCUSSION

Salinity effects on root-shoot length: Root length of the plant samples were taken after 75 days of inoculation (Fig. 1a). Sugarcane plants showed maximum root length (591.75 cm) in autoclaved soil with *Bacillus*, followed by un-autoclaved soil with *Bacillus* (511.75 cm) which is significantly higher from their controls (unautoclaved soil-440.45 cm and autoclaved soil- 425.80 cm). In soil supplemented with 1% NaCl, root length reduced to 250.10 cm in unautoclaved and 212.80 cm autoclaved soils, respectively. However supplementation of *Bacillus* inoculum to unautoclaved and autoclaved soil amended with 1% NaCl, promoted the root growth significantly (320.60 and 267.60 cm). Plants had better root growth in soil amended with 2% NaCl

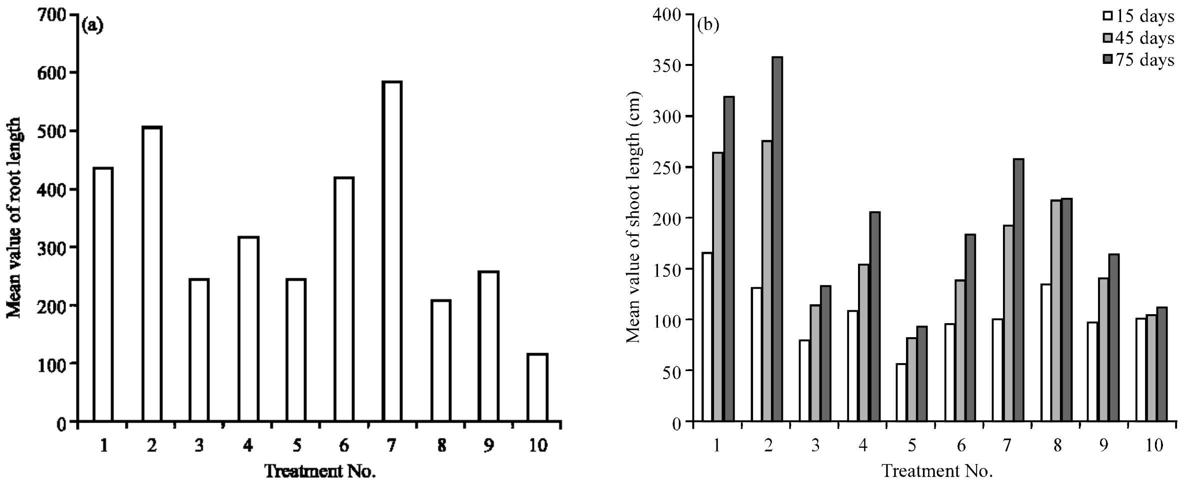

Fig. 1(a-b): Effect of *Bacillus* inoculum on sugarcane root and shoot No. under salinity stress. The data is mean of three independent studies, (a) Root length after 75 days of inoculation and (b) Shoot length. The samples were taken after 15, 45 and 75 days of inoculation. Treatment No.: (1) Unautoclaved soil, (2) Unautoclaved soil+*Bacillus*, (3) Unautoclaved soil+1.0% NaCl, (4) Unautoclaved soil+1.0% NaCl+*Bacillus*, (5) Unautoclaved soil+2.0% NaCl+*Bacillus*, (6) Autoclaved soil, (7) Autoclaved soil+*Bacillus*, (8) Autoclaved soil+1.0% NaCl, (9) Autoclaved soil+1.0% NaCl+*Bacillus* and (10) Autoclaved soil+2.0% NaCl+*Bacillus*

in presence of *Bacillus* in unautoclaved soil (155.65 cm) compared to autoclaved samples (121.55 cm) (Fig. 1a). While during shoot length analysis, after an initial reduction in shoot length at 15th day in both unautoclaved (from 163.56 to 132.10 cm) and autoclaved (from 135.00 to 101.90 cm) soils we observed positive growth when supplemented with *Bacillus* and negative growth with NaCl supplementation (Fig. 1b). Noticeably, unautoclaved soil with *Bacillus* inoculum was found more effective than autoclaved soil with inoculum, since 1% NaCl treated unautoclaved soil showed higher shoot length increase, i.e., from 64.30 to 86.03% on adding *Bacillus*. Whereas, 1% NaCl treated autoclaved soil showed shoot length increase from 61.99 to 69.70% on adding *Bacillus*.

The results suggest that *Bacillus* acts like PGPR and promotes root and shoot growth. *Bacillus* alone as well as with other rhizosphere microbes is found beneficial to sugarcane growth under normal and 1% NaCl stress. Supplementation of PGPRs have been reported to enhance root hair formation in several crops and also protect them against salinity stress (Paul and Nair, 2008; Ashrafuzzaman *et al*., 2009; Mia *et al*., 2010). This might be due to the interactive effect of soil organisms that results in higher N_2 fixation, induction of better root growth and to facilitate better absorption of nutrients from soil (Mia *et al*., 2010).

Plant growth and chlorophyll estimation: One of the parameters that symbolize plant growth is leaf number, higher the number of leaves more is the photosynthesis, better the plant growth. Growth rate is found to be significantly influenced by salinity and supplementation of inoculums having PGPR activity. Maximum increase in leaf number was observed in treatment with *Bacillus* in both autoclaved and unautoclaved soil (which also contains other rhizospherial microbes). While

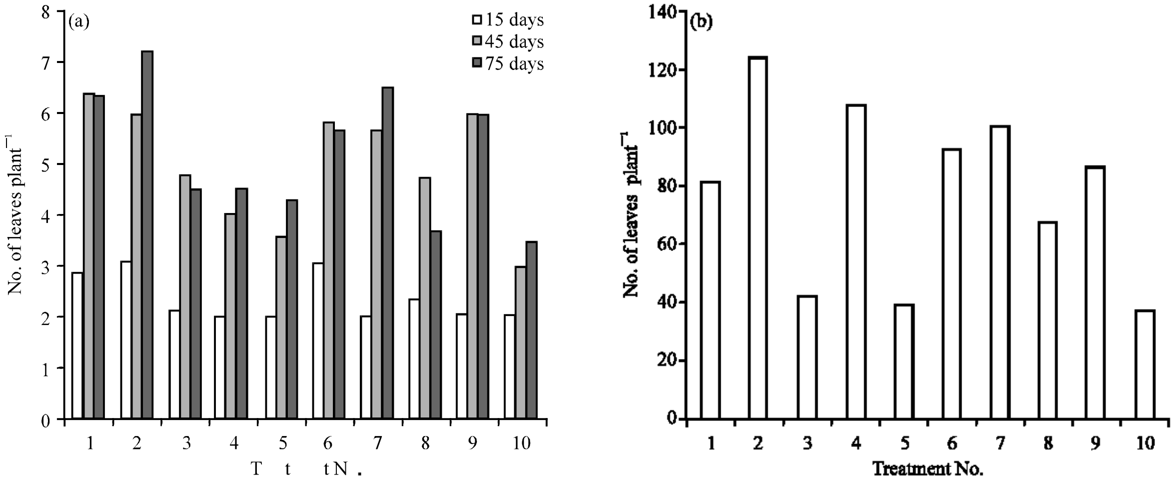

Fig. 2(a-b): Effect of *Bacillus* inoculum on sugarcane leaf No., chlorophyll content under salinity stress. The data is mean of three independent studies, (a) Number of leaves per plant, (b) Leaf Chlorophyll content. Treatment No.: (1) Unautoclaved soil, (2) Unautoclaved soil+*Bacillus*, (3) Unautoclaved soil+1.0% NaCl, (4) Unautoclaved soil+1.0% NaCl+*Bacillus*, (5) Unautoclaved soil+2.0% NaCl+*Bacillus*, (6) Autoclaved soil, (7) Autoclaved soil+*Bacillus*, (8) Autoclaved soil+1.0% NaCl, (9) Autoclaved soil+1.0% NaCl+*Bacillus* and (10) Autoclaved soil+2.0% NaCl+*Bacillus*

in unautoclaved soil with and without NaCl and in autoclaved soil amended with 1% NaCl, a decrease in number of leaves was noticed. Sugarcane seedlings grown in un-autoclaved soil and in presence of *Bacillus* shows maximum increase (2.86 to 6.34 and 3.08 to 7.25, respectively) in leaf number over a period of 75 days of inoculation, followed by autoclaved soil with *Bacillus* (2.02 to 6.5) and with 1% NaCl (2.08 to 6.00). The least was in presence of autoclaved soil with 1% NaCl (2.33 to 3.66) (Fig. 2a). Earlier reports suggests that salinity causes significant reductions in leaf number (Nieves *et al*., 2011) and supplementation of PGPRs ameliorated the deleterious effects of salt stress on plant growth (Yildirim *et al*., 2008; Karakurt and Aslantas, 2010). Adverse effect of salt on the growth of plant was visible in leaf chlorophyll content. The total chlorophyll content was lowest in autoclaved soil with 2% NaCl and *Bacillus* inoculum (mean values 37.58 mg chl g^{-1} of leaf), followed by unautoclaved soils with *Bacillus* and 2% NaCl (39.17 mg chl g^{-1} of leaf) and unautoclaved soil in 1% NaCl without inoculum (42.46 mg chl g^{-1} of leaf) respectively (Fig. 2b). Highest chlorophyll content was observed in unautoclaved soil with *Bacillus* supplementation (123.95 mg chl g^{-1} of leaf).

Reduction of plant growth caused by salinity stress is the most common phenomenon of plants under stress, results from the alteration of many physiological/metabolic processes such as photosynthetic activity, mineral uptake and antioxidant activity (Golpayegani and Tilebeni, 2011). The results of this study indicate positive effect of *Bacillus* inoculum on biomass and chlorophyll content which has relevance to photosynthetic efficiency of the plants. Absence of rhizosphere organisms did not show any significant difference in total chlorophyll content over the *Bacillus* inoculum.

MDA, proline and reducing sugar content: Malondialdehyde (MDA) is a product of peroxidation of unsaturated fatty acids and has been used as an indicator of free radical damage

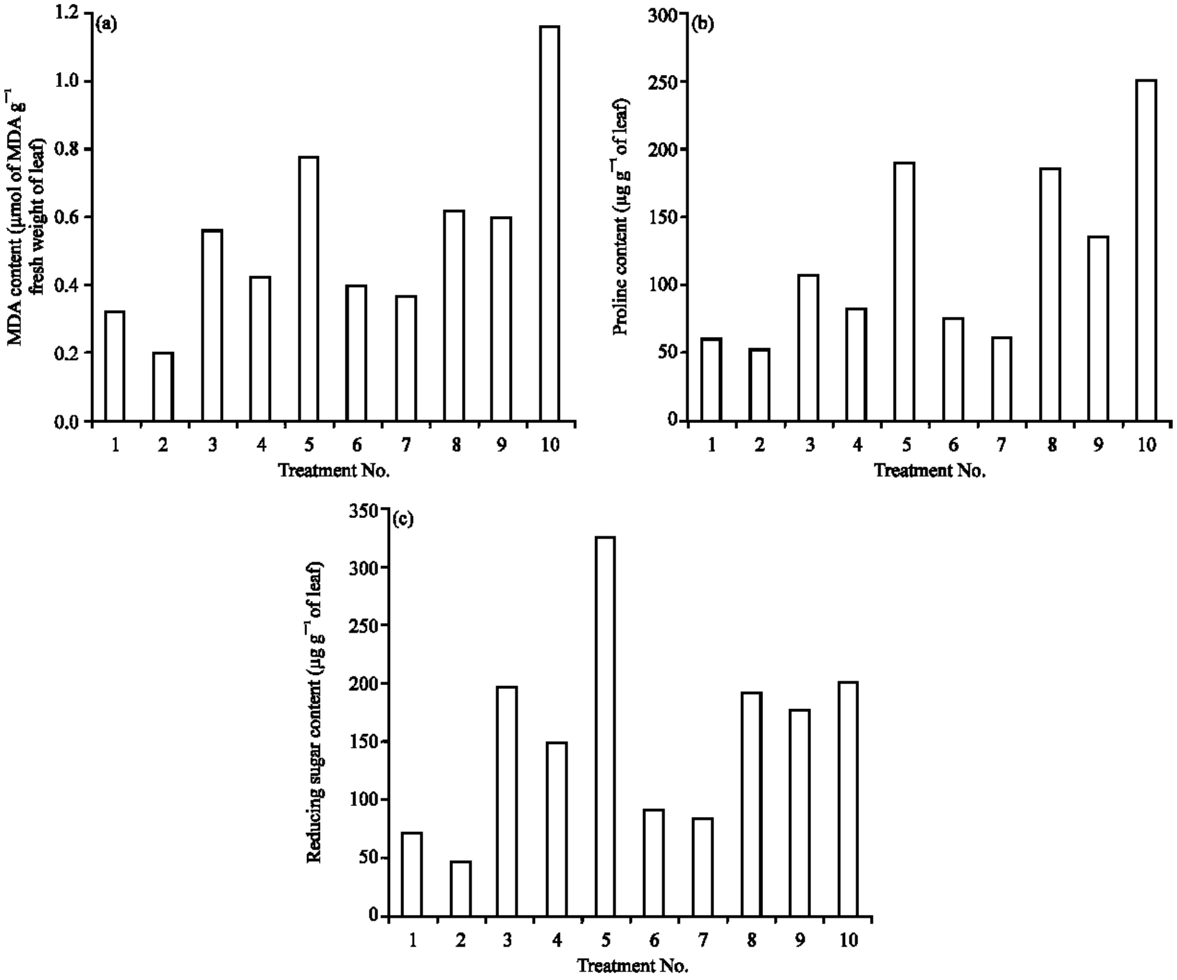

Fig. 3(a-c): Effect of *Bacillus* inoculum on sugarcane leaf MDA, proline, reducing sugar content and *Bacillus* cell count under salinity stress. The data is mean of three independent studies, (a) Leaf MDA content, (b) Leaf proline content and (c) Total leaf sugar content. The samples were taken after 75 days of inoculation. Treatment No.: (1) Unautoclaved soil, (2) Unautoclaved soil+*Bacillus*, (3) Unautoclaved soil+1.0% NaCl, (4) Unautoclaved soil+1.0% NaCl+*Bacillus*, (5) Unautoclaved soil+2.0% NaCl+*Bacillus*, (6) Autoclaved soil, (7) Autoclaved soil+*Bacillus*, (8) Autoclaved soil+1.0% NaCl, (9) Autoclaved soil+1.0% NaCl+*Bacillus* and (10) Autoclaved soil+2.0% NaCl+*Bacillus*

to cell membranes under stress conditions (Jaleel *et al.*, 2007). Maximum MDA content was found in sugarcane plants grown in 2% NaCl supplemented autoclaved soil with *Bacillus* inoculum (1.16 µmol of MDA g^{-1}) followed by un-autoclaved soil in 2% NaCl and *Bacillus* (0.78 µmol of MDA g^{-1}) (Fig. 3a) and least was in unautoclaved soil with *Bacillus* inoculum (0.20 µmol of MDA g^{-1}). MDA content of the samples with *Bacillus* inoculum was lower with respect to their control. Increased lipid peroxidation was observed in plants subjected to salinity stress. Supplementation of PGPR like *Bacillus*, reduces MDA content in plants grown in salinity stress and play a significant role in preventing the abiotic stress induced oxidative damage in plants (Arora *et al.*, 2008; Borzouei *et al.*, 2012; Azooz *et al.*, 2009) (Fig. 3).

Proline acts as compatible solute in osmotic adjustment, enzyme protectant, stabilizes membranes and cellular structures during hostile conditions (Al-Taha, 2013; Padmavathi and Rao, 2013). Plants grown on soil amendment with 1%NaCl showed enhanced leaf proline content in both unautoclaved (107.00 μg g^{-1}) and autoclaved sample (185.65 μg g^{-1}) over their respective control (53.11 and 61.39 μg g^{-1}, Fig. 3b). Higher leaf proline content was noticed in autoclaved and unautoclaved samples supplemented with 2% NaCl and *Bacillus* inoculum (250.87 and 190.09 μg g^{-1}, respectively). *Bacillus* inoculum reduced proline content in plants grown in NaCl amended unautoclaved soil (107.00 to 82.63 μg g^{-1}) and autoclaved soil (185.65 to 136.62 μg g^{-1}). Increase in proline content with salinity could be due to (1) Breakdown of proline rich proteins, (2) Prevention of feedback inhibition of biosynthetic enzyme by sequestering proline from its site of synthesis and (3) Decreased activity of enzymes involved in degradation of proline such as proline dehydrogenase and proline oxidase (Summart *et al.*, 2010) (Fig. 3).

Higher leaf reducing sugar was observed in plants grown in autoclaved soil than un-autoclaved soil (Fig. 3c). With *Bacillus* supplementation, leaf reducing sugar was reduced in plants grown in autoclaved (91.81 to 83.62 μg glucose g^{-1}) and un-autoclaved (71.35 to 47.33 μg glucose g^{-1}) soils. Amending the autoclaved and un-autoclaved soil with 1% NaCl, enhanced leaf reducing sugar content (193.06 and 197.33 μg sugar g^{-1}, respectively), which was significantly reduced (177.69 and 149.82 μg sugar g^{-1}) with *Bacillus* inoculum. Accumulation of sugars has been associated with drought and salinity tolerant mechanisms. Increase in leaf sugar content under salt stress has been reported in soybean (Sobhanian *et al.*, 2010) and mangroves (Naureen and Naqvi, 2010).

Protein content, ascorbate peroxidase and catalase activity: Proteins participate in various cellular metabolic processes. Level of proteins, antioxidant enzymes and antioxidants are frequently used as indicators to measure oxidative stress in plants. Leaf soluble protein was found to be highest in plants grown in autoclaved soil with 2% NaCl and *Bacillus* inoculum (998.91 μg g^{-1}) followed by autoclaved soil with 1% NaCl (875.13 μg g^{-1}, Fig. 4a). Least leaf soluble protein content was found in unautoclaved soil with *Bacillus* inoculum (115.79 μg g^{-1}). Plants grown on soil (both autoclaved, 869.07 μg g^{-1} and unautoclaved, 757.37 μg g^{-1}) amendment with NaCl showed high leaf soluble protein content compared to their respective controls (429.94 and 319.90 μg g^{-1}, respectively). *Bacillus* inoculum significantly reduced the leaf soluble protein content (640.01 and 553.36 μg g^{-1}, respectively).

Maximum catalase (15.11 units mg^{-1} protein) and ascorbate peroxidase (81.03 units mg^{-1} of protein) activity was observed in autoclaved soil with 2% NaCl and *Bacillus*. Significant reduction of leaf ascorbate peroxidase and catalase activity was observed in unautoclaved (1.87 and 5.79 to 4.24 units mg^{-1} of protein, respectively.) and autoclaved soil (19.22 to 16.91 and 7.26 to 6.58 units mg^{-1} of protein, respectively) with *Bacillus* inoculum. Activities of both the enzymes were significantly higher in autoclaved soil amended with 1% NaCl (62.35 and 14.24 units mg^{-1} of protein) over their control (39.11 and 8.46 units mg^{-1}). These results indicate that NaCl stresses induce/enhance the activity of catalase and ascorbate peroxidase to scavenge/alleviate the ROS mediated toxicity (Fig. 4b, c). Amending with *Bacillus* inoculum seems to protect plants from salinity induced stress and activity both enzymes reduced significantly in normal as well as NaCl amended soils. Rhizosphere microbes and *Bacillus* together helps the plants to survive salinity stress, probably by diverting plant metabolic processes to detoxify as well as to mitigate the production of ROS *in vivo* (Sharma *et al.*, 2012).

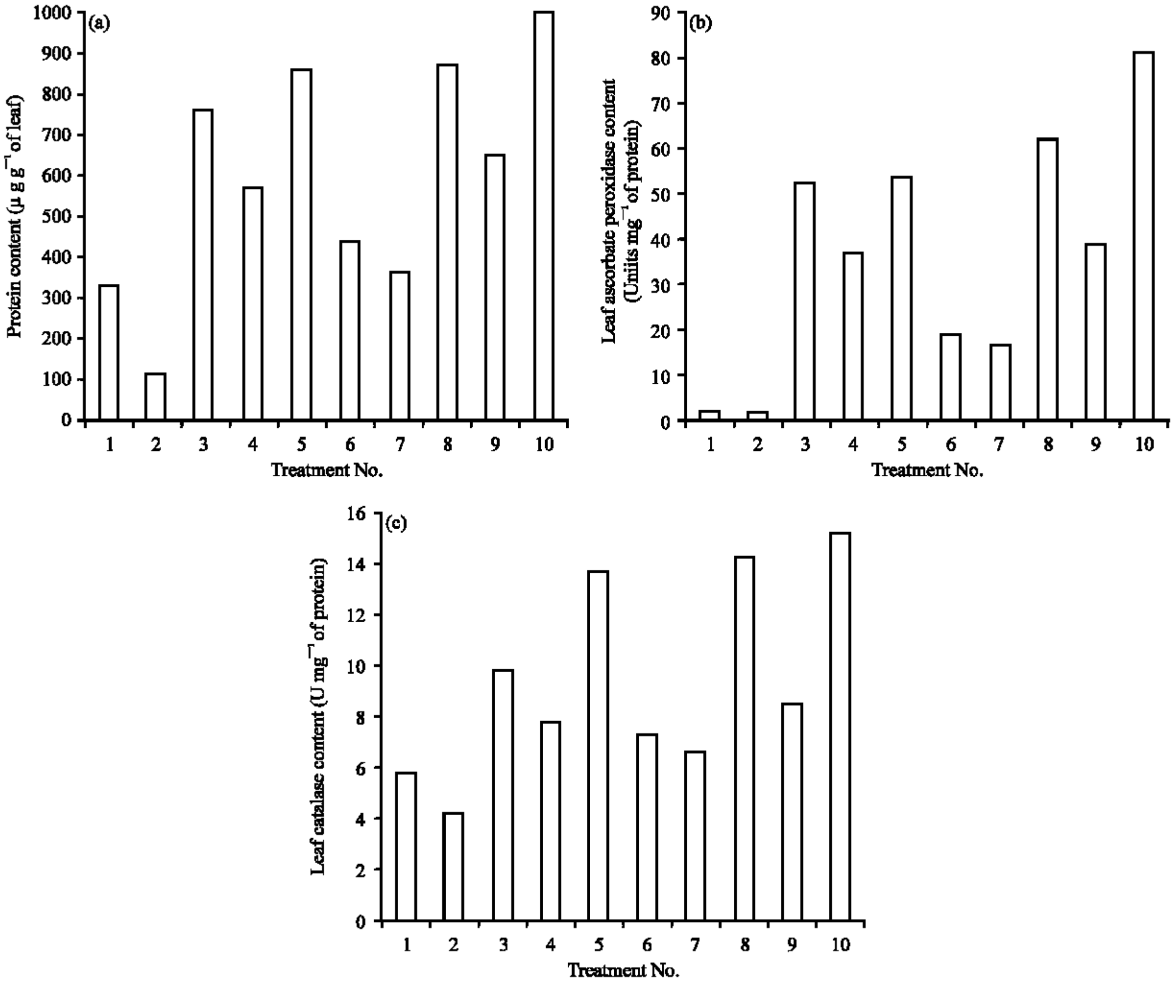

Fig. 4(a-c): Effect of *Bacillus* inoculum on sugarcane leaf protein, ascorbate peroxidase and catalase activity under salinity stress. The data is mean of three independent studies, (a) Total leaf soluble protein, (b) Leaf ascorbate peroxidase activity, (c) Leaf catalase activity. The samples were taken after 75 days of inoculation. Treatment No.: (1) Unautoclaved soil, (2) Unautoclaved soil+*Bacillus*, (3) Unautoclaved soil+1.0% NaCl, (4) Unautoclaved soil+1.0% NaCl+*Bacillus*, (5) Unautoclaved soil+2.0% NaCl+*Bacillus*, (6) Autoclaved soil, (7) Autoclaved soil+*Bacillus*, (8) Autoclaved soil+1.0% NaCl, (9) Autoclaved soil+1.0% NaCl+*Bacillus* and (10) Autoclaved soil+2.0% NaCl+*Bacillus*

Cation content: Accumulation of sodium in leaves was significantly higher in plant grown on both NaCl amended unautoclaved (0.19 to 0.46%) and autoclaved (0.21 to 0.60%) soil. Similar trend was also observed in soils with *Bacillus* and NaCl amended unautoclaved and autoclaved soils. However in *Bacillus* treated samples, a significant reduction in the accumulation of leaf sodium was recorded in unautoclaved and autoclaved control (0.19 to 0.08%; 0.21 to 0.18%) as well as NaCl (0.46 to 0.29%; 0.60 to 0.31%) amended soils (Fig. 5a). These results suggest that *Bacillus* inoculum protects the plants from large accumulation of Na in leaves in control and salinity stressed plants. Contrastingly, plants grown on saline soils accumulated less leaf potassium compared to their controls and were reverse in plants supplemented with *Bacillus* inoculum. Leaf potassium

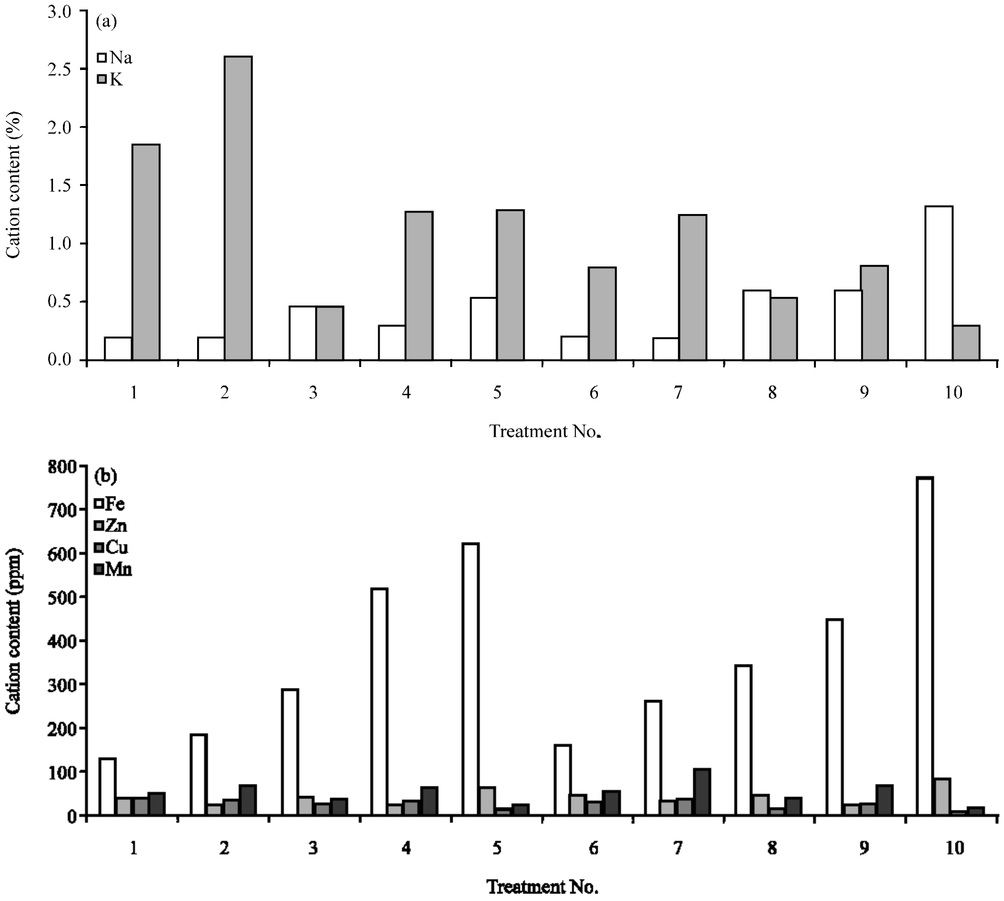

Fig. 5(a-b): Effect of *Bacillus* inoculum on sugarcane leaf cation content under salinity stress. The data is mean of three independent studies, (a) Sodium, Potassium and (b) Iron, Copper, Manganese and Zinc. Samples were taken at 75 days of inoculation. Treatment No.: (1) Unautoclaved soil, (2) Unautoclaved soil+*Bacillus*, (3) Unautoclaved soil+1.0% NaCl, (4) Unautoclaved soil+1.0% NaCl+*Bacillus*, (5) Unautoclaved soil+2.0% NaCl+*Bacillus*, (6) Autoclaved soil, (7) Autoclaved soil+*Bacillus*, (8) Autoclaved soil+1.0% NaCl, (9) Autoclaved soil+1.0% NaCl+*Bacillus* and (10) Autoclaved soil+2.0% NaCl+*Bacillus*

content significantly higher in *Bacillus* supplemented unautoclaved soil (2.61%) over its control (1.86%), however in NaCl amended soil it reduced to 1.29 and 0.46%, respectively. Leaf potassium content in plants grown in autoclaved soil increased from 0.80 to 1.26% in *Bacillus* inoculated sample; however it reduced significantly (0.55 and 0.80%, respectively; Fig. 5b).

Leaf iron content was significantly high in unautoclaved soil with *Bacillus* inoculum (772.60 ppm), compared to its control unautoclaved soil (621.00 ppm, Fig. 5b). Plants grown on autoclaved soil showed lower accumulation of iron (450.70 ppm), which was further reduced in both unautoclaved and autoclaved soil amended with NaCl (260.30 and 186.50 ppm). However, there was recovery in the accumulation of iron with *Bacillus* inoculum (347.00 and 288.50 ppm) in NaCl amended soils. In contrast, zinc accumulation was higher in unautoclaved and autoclaved soil amended with 2% NaCl and *Bacillus* (69.00 and 88.00 ppm) followed by soils amended with

1%NaCl (46.00 and 50.00 ppm). A reduction in the leaf zinc accumulation was observed in unautoclaved (40.20 to 26.70 ppm) and autoclaved (47.70 to 36.00 ppm) soil with *Bacillus* inoculum alone. Accumulation of copper in leaves was found to be significantly lower in unautoclaved (17.33 ppm) and autoclaved (13.40 ppm) soil amended with 2% NaCl and *Bacillus*. However in all the treatments, supplementation of *Bacillus* inoculum facilitated the leaf copper accumulation to certain extent (Fig. 5b). NaCl amendment significantly reduced the accumulation of manganese in plants grown in unautoclaved and autoclaved soils. However amending the soil with *Bacillus* enhanced the accumulation of manganese in control (Unautoclaved 27.70 to 35.45 ppm and autoclaved 35.30 to 38.68 ppm) and in presence of NaCl (Unautoclaved 28.89 to 33.39 ppm and autoclaved 20.83 to 26.26 ppm), suggests that *Bacillus* facilitate the uptake of manganese in sugarcane plants (Fig. 5b).

Saline conditions drastically change the environment of root aeration, osmotic potential of soil solution and normal equilibrium of dissolved ions. The availability of micronutrients to crop plants mainly depends on pH of the soil solution. In saline and sodic soils, the solubility of micronutrients (Cu, Mn, Fe and Zn) is particularly low and plants growing on such soils often experience nutrient deficiency (Achakzai *et al.*, 2010). *Bacillus* along with other rhizosphere organisms was found helpful in providing suitable ecosystem in the roots to facilitate the acquisition of cat ions required for plant growth and development under adverse situation viz., salinity stress.

CONCLUSION

Sugarcane represents an important renewable source among biofuel crops with high capability to assimilate carbon among the C4 plants. Limited availability of fresh water and presence of high salt, render this crop uneconomical, warranting the necessity for the development of varieties with higher water use efficiency and tolerant to salt stress. Plants adapt to stress in order to survive and respond by altering the various biochemical and physiological processes. In plants, roots are the primary target, which perceives the stress. In the root rhizosphere, some plants promote symbiotic relationship with organisms to acquire stress tolerance. *Bacillus subtilis* isolated from the sugarcane root rhizosphere was found to facilitate and improve the growth of sugarcane plants grown with salinity stress. *Bacillus* in presence of other rhizospheric microbes showed better growth response and also helped the buds to sustain under saline stress. Significantly, sugarcane plants did not survive in soil amended with 2% NaCl but addition of *Bacillus* inoculum helped them same to survive and grow. The results put suggest that *Bacillus subtilis* isolated from the sugarcane root rhizosphere can be a very good bioinoculum to alleviate the salinity stress when sugarcane is grown arable land turning to saline rich patches due to extensive agriculture and possibly boon to sugarcane farmers in the country.

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