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Studies of Plant Growth Promoting Rhizobacterial Inoculants on Sugarcane in Saline Soil

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Abstract

Salinity of soil is an emerging problem of the agriculture that reduces plant growth and yield. Use of plant growth promoting rhizobacteria (PGPR) inoculant in saline soil improves fertility and induces plant growth promotion. These beneficial microorganisms colonize the rhizosphere /endo-rhizosphere of plants and impart saline tolerance. Halo tolerant PGPR cultures were isolated from saline soil fields based on soil's physico-chemical properties from Baramati region. The selected isolates *Azotobacter* spp., *Rhizobium* spp. and *Azospirillum* spp. were characterized on the basis of morphological and biochemical tests. These cultures were salt tolerant up to 2 % NaCl and having nitrogen fixation, alkaline phosphatase, indole acetic acid (IAA) and exopolysaccharide production activity. We assessed PGPR inoculants on sugarcane grown in saline soil by pot assay method. This method carried out by giving treatment to saline soil with halo tolerant PGPR inoculants. Sugarcane plantlets germination rate, shoot length, chlorophyll content of leaf and percent nitrogen content of leaf improved in pots inoculated with of halo tolerant PGPR inoculants. Simultaneously, all halo tolerant PGPR inoculants improved saline soil health in treated pot soil over control, with respect to available nitrogen, phosphorus, potassium and organic carbon also decreasing electrical conductivity, pH and sodium adsorption ratio of saline soil. The present article focuses on evaluation of halo tolerant bacterial strains to stimulate saline tolerance and promote growth of sugarcane in saline soil. It inferred that PGPR inoculants are applicable in promoting plant growth under salt stress.

Significance Statement:

Sugarcane is the major crop of farmers. Salinity of soil affects growth and productivity of sugarcane. This study was conducted in an attempt to isolate and characterize halo tolerant PGPR from saline soil habitat and its efficacy in it.

Keywords

Saline soil, halo tolerant PGPR, sugarcane, pot assay.

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INTRODUCTION:

Salinity of agriculture soil is one of the most common environmental stress factors that adversely affect plant productivity by retarding plant growth and development. The overuse of water and chemical fertilizers has plays significant role in increasing

salinization of soil. One of the major complications in this process is the increase in the concentration of soluble salts in the root zone of soils, which affects the rhizospheric populations thereby affecting plant productivity [1]. Soil salinity limits the lands capability for supporting optimum plant growth therefore growing demands of expanding population for various biomass products have necessitated an exploitation of these soils [2]. A new biological approach of plant microbe interaction to conquer salinity troubles has recently gained a great interest from many workers throughout the world. Use of rhizobacteria is one of the most acceptable approach to reduce the effect of salt stress on plants by mechanisms which either modulate or ameliorate the salt stress [3].Soil organic matter and beneficial soil microbes have been recognized as key factor in maintaining soil quality and crop production. Bioinoculants contain beneficial microbes that enhance plant growth when applied in soil by nutrient solubilization, nitrogen fixation, phytohormones production resulting in available forms of nutrients in soil which improved soil properties and productivity [4,5].To make agriculture sustainable and less dependent on chemical fertilizers it is important to know how to use PGPR that can biologically fix nitrogen, solubilize phosphorus and induce IAA that can contribute to improvement of crop growth.

Plant growth promoting rhizobacteria (PGPR) can protect plants from deleterious effects of environmental stresses including drought, salinity, heavy metal and phytopathogens. Many plant growths promoting rhizobacteria (PGPR) facilitate plant growth indirectly by reducing plant pathogens or directly by facilitating the uptake of nutrients from environment. PGPR influence the plant hormonal balance by producing compound such as phytoharmone indole acetic acid. They can mobilize nutrients to plants such as phosphorus by solubilization of soil insoluble phosphates. Some rhizobacteria produce microbial inhibitory compounds such as siderophore Fe chelating molecules that inhibit growth of phytopathogen in soils with low content of this ion promoting indirectly the plant growth. PGPR fixes nitrogen from environment that becomes available to plants [3]. To rescue plant growth in saline conditions, PGPR have been known to play an essential role in the growth and metabolism of plants [6]. Certain varieties of PGPR *Bacillus*, *Burkholderia*, *Acenitobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Beijerinckia*, *Flavobacterium*, *Rhizobium* and *Serratia* are now being used worldwide as biofertilizer to enhance crop productivity [5,7]. Strains from *Azospirillum*, *Bacillus*, *Azotobacter* are commercialized as biofertilizers for non-legumes plants. There is no commercial biofertilizer for non-legumes based on *Rhizobium*. But *Rhizobium* has potential as non-legume plant growth promotion by producing IAA, phosphate solubilization, exopolysaccharide production and siderophore production. *Rhizobium* promotes the growth of non-leguminous plants like sunflower, canola, tomato, pepper shown in other reports [8, 9]. So, we selected *Rhizobium* as PGPR inoculants in this study.

Today, much of agriculture land in Maharashtra has become saline due to faulty irrigation practices and overuse of chemical fertilizers. Sugarcane is the major crop of farmers. Salinity of soil effect on growth and productivity of sugarcane. This study was conducted in an attempt to isolate and characterize halo tolerant PGPR from saline soil habitat and to evaluate their ability of improvement in saline soil properties and sugarcane plant growth promotion in saline soil by pot assay method.

MATERIAL AND METHODS:

Sample collection:

Baramati Tehsil region, Maharashtra, India was chosen for sample collection. The locations were Dorlewadi, Zargardwadi, Malegaon,Medad, Shardanagar, Krishi Vigyan Kendra Malegaon, Songaon. A total of 50 saline sites were chosen from the locations mentioned. Soil with pH higher than 8.5 and electrical conductivity above 2.5 dS/m were chosen for the study. From each saline site at least 60cm deep soil was taken. Soil samples were collected from the rhizosphere area of plants. The soil samples were placed in plastic bags and stored at room temp. At selected point in the trial area without bulking sample, because soil is spatially variable. For *Rhizobium* strains roots of leguminous plants were removed. All the samples were taken in different polythene bags and brought to the laboratory [10].

Isolation and identification of PGPR cultures

Enrichment of organism carried out in Ashby's broth and yeast extract mannitol broth. All bacteria were

isolated on yeast extract mannitol agar and Ashby's mannitol agar media. Isolates biochemically characterized by Gram's staining, motility and biochemical tests like catalase, oxidase, sugar utilization, ammonia production, amylase test and citrate utilization tests were performed as per standard methods [11]. All isolates were identified as per the Bergey's Manual of Determinative Bacteriology 9th Edition [12]. Specific medium for *Azotobacter* spp. Ashby's mannitol media, *Rhizobium* spp. yeast extract mannitol media and *Azospirillum* spp. medium for *Azospirillum* used for inoculants production.

Determination of salt tolerance

Isolated cultures were screened for salt tolerance. These cultures were grown in specific medium broth supplemented with NaCl so to give 0.4-2% NaCl concentration. Each tube was then added with actively growing selected PGPR and incubated on rotary shaker at 30 °C. Bacterial growth was determined as OD_{540} to find out NaCl tolerance.

Abbreviations: PGPR=Plant Growth Promoting Rhizobacteria, IAA=Indole Acetic Acid, AN= Available Nitrogen, AP= Available Phosphorus, AK= Available Potassium

Characterization of PGPR for plant growth promotion traits

Production of Indole acetic acid

The isolates were tested for production of growth hormone i.e. auxins (IAA). The bacterial cultures were inoculated in Jenson's broth (0.5g of Tryptophan for 100ml media). Incubation was done at 28^{0}C for 7 day at 100 rpm on orbital shaking incubator. After completion of incubation days the broths were centrifuged at 10,000 rpm for 15min at 4^{0}C. 2 ml supernatant was taken and 2 drops of orthophosphoric acid and 4ml of Salkowasky's reagent was added. Pink color production indicated IAA production. Absorbance was measured at 530nm. The absorbance was compared with standard curve and the concentration of IAA produced was calculated accordingly [13].

Phosphate solubilization in liquid culture (Alkaline phosphatase activity):

Isolates were grown in selective media. One ml of culture supernatant was incubated at room temperature with 1.0 ml of 25 mM q-nitro phenyl phosphate and 4.0 ml modified universal buffer, pH 11, alkaline phosphatase. After 1 hour the reaction was terminated by adding 1.0 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH. The assay mixtures were filtered through a Whatman No. 2 filter paper and the yellow color measured at 410 nm. A standard curve drawn from known concentration of q-nitrophenyl phosphate was used to quantify alkaline phosphatase activity present in the culture supernatant.

Nitrogen fixation

PGPR cultures were tested for nitrogen fixation in Ashby's broth nitrogen free medium. Inoculation of PGPR culture in Ashby's broth incubated at 28^{0}C-30^{0}C for 7-8 days then observed it for turbidity formation.

Exopolysaccharide production

PGPR isolates were grown on selective media broth. Cell mass was removed from 30 days old cultures broth by centrifugation (10,000 rpm) for 10 min at 20°C. In 20 ml supernatant, double volume ice cold isopropanol was added and kept overnight at 4°C. The precipitated polysaccharides were separated by centrifugation (10,000 rpm) and dried in pre weighed porcelain dish which were kept in the oven. Extracellular polysaccharide content (mg/ml) was determined from the dry weights of cell extract.

Soil physiochemical analysis

Saline soil samples were analyzed for physicochemical parameters like pH, electrical conductivity, total organic carbon, total nitrogen, phosphorus content and potassium content by standard methods [14].

Pot assay

PGPR liquid inoculants of *Azotobacter* spp., *Rhizobium* spp. and *Azospirullum* spp. were prepared in their specific medium with the cell population adjusted to 1×10^8 - 1×10^9cfu/ml determined by standard plate count method. Efficacy of inoculants was studied by pot assay with sugarcane variety co-86032(*Saccharum officinarum*) as a test crop. Eight treatments in triplicate were used. Three bacterial cultures which are *Azotobacter* spp. (AZT), *Rhizobium* spp. (RZB) and *Azospirullum* spp. (AZSP) were used treatments are AZT+RZB, AZSP+RZB, AZT+AZSP, AZT+RZB+AZSP and control [5]. Saline soil collected from salt affected field

used for pot assay. 5 kg saline soil was added in each earthen pot and saline soil was treated with PGPR inoculants as per the treatment given in the table, 300ml per 5 kg soil or 100 ml of each inoculant for consortia treatment kept it for one day. Sugarcane eye buds surface sterilized with 0.1% $HgCl_2$ and washed with water before using. In each pot sugarcane eye buds sown at 5 cm depth as four buds in each pot. The moisture content maintained by irrigating pots 1-day interval.

Table 1 Details of the treatments for sugarcane pot assay

Sr. No.	Treatment code	Treatment forms	Short Treatments details
1	T1	SS	Saline soil as a control
2	T2	SS+AZT	Saline soil + *Azotobacter* spp.
3	T3	SS+RZB	Saline soil + *Rhizobium* spp.
4	T4	SS+AZSP	Saline soil + *Azospirillum* spp.
5	T5	SS+AZT+RZB	Saline soil + *Azotobacter* spp. + *Rhizobium* spp.
6	T6	SS+AZSP+RZB	Saline soil + *Azospirillum* spp. + *Rhizobium* spp.
7	T7	SS+AZT+AZSP	Saline soil + *Azotobacter* spp. +*Azospirillum* spp.
8	T8	SS+AZT+RZB+AZSP	Saline soil + *Azotobacter* spp. + *Rhizobium* spp. + *Azospirillum* spp.

Sugarcane pot assay

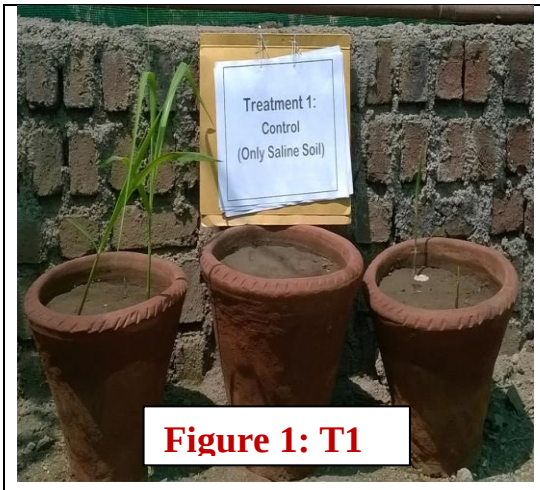

Figure 1: T1

Figure 2: T2

Figure 3: T3

Figure 4: T4

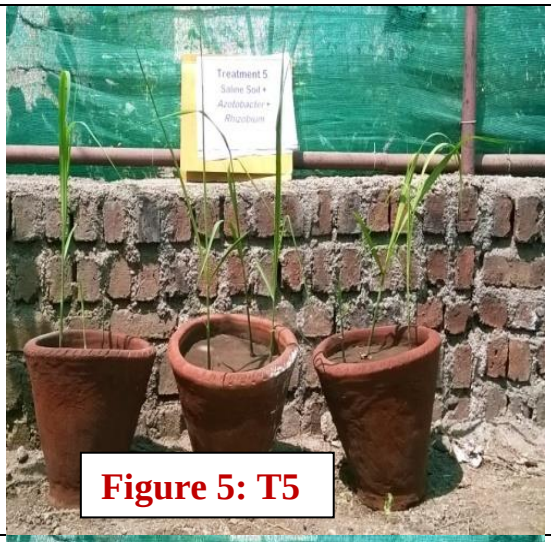

Figure 5: T5

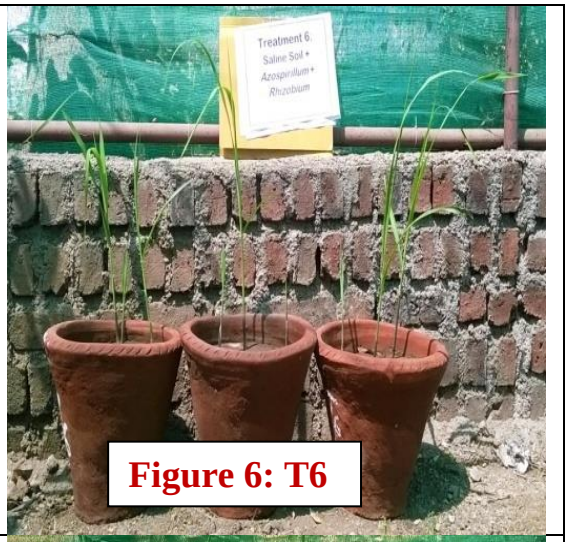

Figure 6: T6

Figure 7: T7

Figure 8: T8

Treated pots were analyzed for their soil properties and plant growth parameters after 45 day after sowing. Analyzed for parameters given table 2

Table 2 Parameters of Plant growth and soil properties

Plant Growth	Soil Properties
1. Germination (%)	1. pH
2. Shoot Length (cm)	2. Electrical conductivity (dS/m)
3. Shoot Fresh weight(g)	3. Sodium adsorption ratio (SAR)
4. Total Chlorophyll content (mg/g)	4. Sodium content (mEq/L)
5. Total Nitrogen content of leaf (%)	5. Available Nitrogen (mg/kg)
	6. Available Phosphorus (mg/kg)
	7. Available Potassium (mg/kg)
	8. Organic Carbon (%)
	9. Total Nitrogen (%)

Statistical analyses:

By 2^3 factorial design total eight treatments were designed for this experimentation. The statistical model was developed by applying multiple regression analysis using the obtained experimental data for plant growth, salinity parameters and nutrient content of soil. Regression coefficient was determined to predict the accuracy of the model. A 95% confidence interval was used.

RESULTS:

Altogether 25 pure cultures of plant growth promoting rhizobacteria were isolated from saline soil field from Baramati region and tested for their nitrogen fixing potential, IAA production and phosphate solubilization potential. Based on such data, three potent isolates were selected for further study

Morphology and biochemical characteristics:

All isolates were identified as per the Bergey's Manual of Determinative Bacteriology 9th Edition. The bacterial isolates were identified as *Rhizobium* spp., *Azotobacter* spp. and *Azospirullum* spp. All isolates are Gram negative and motile. *Rhizobium* spp. was ribose, xylose, mannose, galactose, arabinose maltose and lactose positive. *Azospirullum* spp. *was* oxidase positive utilizes glucose, α ketoglutarate and citrate. *Azotobacter* spp. is catalase positive.

Effect of Salt concentration on microbial growth:

PGPR cultures were screened for the salt tolerance (0.4% to 2% NaCl). Isolates were shown tolerance to 2.0 % NaCl concentration.

Plant growth promoting activities of PGPR

In this study cultures were screened for synthesis of IAA production in the presence of L-tryptophan as precursor. All selected isolate produced IAA ranging from 11-103 µg/ml in selective media broth. Culture *Azospirillum* spp. showed maximum production of IAA (103 µg/ml). Selected cultures were shown positive alkaline phosphatase activity. *Rhizobium* spp. culture showed maximum alkaline phosphatase activity (240µM/ml/h). Isolates were grown in nitrogen free Ashby's medium. .*Azotobacter* spp. and *Azospirillum* spp. showed growth in nitrogen free Ashby's medium. Extracellular polysaccharide content (mg/ml) was determined from the dry weight of cell extract. *Azotobacter* spp. and *Rhizobium* spp. isolates synthesized extracellular polysaccharide. Culture *Azotobacter* spp. showed maximum production of extracellular polysaccharide (3.1 mg/ml).

Effect of PGPR inoculants on saline soil parameters in sugarcane pot assay

Table 4: Effect of inoculants on pH, electrical conductivity, sodium adsorption ratio and sodium content of saline soil

Treatment	pH		EC (dS/m)		SAR		Na (mEq/l)	
	45days	90days	45days	90days	45days	90days	45days	90days
T1	8.8 (0.1)	8.68 (0.13)	3.3 (0.1)	3.24 (0.11)	14.19 (0.28)	13.87 (0.28)	85.15 (1.52)	82.82 (1.58)
T2	8.6 (0.1)	8.42 (0.19)	2.97 (0.14)	2.78 (0.06)	9.83 (0.21)	9.1 (0.22)	66.48 (1.52)	61.82 (1.58)
T3	8.66 (0.05)	8.5 (0.15)	3.06 (0.15)	2.9 (0.03)	10.36 (0.2)	9.9 (0.27)	70.15 (1.52)	67.22 (1.81)
T4	8.53 (0.05)	8.4 (0.15)	2.87 (0.05)	2.72 (0.04)	9.59 (0.24)	8.92 (0.22)	65.15 (1.52)	60.82 (1.58)
T5	8.56 (0.15)	8.46 (0.2)	2.83 (0.047)	2.74 (0.03)	9.98 (0.13)	9.06 (0.22)	67.82 (1)	61.82 (1.58)
T6	8.5 (0.1)	8.36 (0.16)	2.81 (0.06)	2.73 (0.05)	9.46 (0.23)	8.6 (0.27)	64.48 (1.52)	59.02 (1.92)
T7	8.43 (0.05)	8.26 (0.15)	2.76 (0.05)	2.65 (0.04)	8.93 (0.22)	8.36 (0.15)	61.15 (1.52)	57.42 (1.14)
T8	8.3 (0.1)	8.18 (0.13)	2.7 (0.04)	2.47 (0.07)	8.3 (0.15)	7.89 (0.15)	57.82 (1)	55.22 (1.14)

a)EC: Electrical conductivity b)SAR: Sodium Adsorption Ratio c)Na-Sodium; values given in table are of 'mean' of five replicates and values in parentheses are of standard deviations

Analysis of soil properties of experimental soil were carried out after 45 and 90 day of sowing.

The pH, electrical conductivity, sodium adsorption ratio, and sodium content are the soil parameters which highlighted the soil salinity. The pH of control (untreated) soil was 8.8±0.1, where as in PGPR inoculants treatment T8 (pH 8.18±0.13) after 90 days shows higher decrease in the pH of saline soil closely followed by other inoculants treatment shows decrease in pH of saline soil as compare to control soil. Electrical conductivity of soil treated with formulation of treatment eight shows highest decrease after 45 and 90 days T8 EC (2.47 dS/m ±0.07) and other inoculants treatment shows decrease in EC of saline soil as compare with control.

Sodium adsorption ratio of saline soil treated with inoculants was recorded after 45 and 90 days lowest for T8 (7.89±0.15) closely followed by other inoculants treatments shows decrease in SAR as compared with control. The sodium salt is the one of the major content to increase the salinity of soil. We estimate the soluble sodium content of the saline soil in control and soil treated with PGPR inoculants we observe that after 45 and 90 days there was decrease in sodium content of PGPR treated saline soil highest decrease in T8 (Na 55.22 mEq/l ± 1.14) compare to control.

Table 5: Effect of PGPR inoculants on available nitrogen, available phosphorus, available potassium, organic carbon and total nitrogen content of saline soil

Treatment	AN (mg/kg)		AP (mg/kg)		AK (mg/kg)		Org-C (%)		T-N (%)	
	45days	90days	45days	90days	45days	90days	45days	90days	45days	90days
T1	27.62 (1.71)	26.88 (1.77)	21.06 (0.2)	19.58 (0.94)	30.43 (1.52)	29.1 (1.58)	0.34 (0.018)	0.36 (0.038)	0.14 (0.01)	0.12 (0.01)
T2	37.7 (1.71)	39.87 (1.27)	23.6 (0.19)	26.4 (0.54)	40.43 (1.52)	43.7 (1.81)	0.45 (0.021)	0.59 (0.018)	0.41 (0.02)	0.43 (0.01)
T3	28.74 (1.71)	28.0 (1.77)	23.75 (0.35)	28.07 (0.38)	35.76 (1.52)	39.7 (1.67)	0.42 (0.009)	0.63 (0.022)	0.15 (0.02)	0.14 (0.02)
T4	38.08 (1.12)	44.352 (1.27)	24.74 (0.71)	28.77 (0.5)	39.76 (1.52)	44.5 (1.14)	0.38 (0.014)	0.56 (0.03)	0.42 (0.02)	0.44 (0.01)
T5	31.36 (1.12)	34.94 (1.46)	28.95 (0.53)	29.4 (0.48)	38.1 (1)	40.3 (1.3)	0.52 (0.038)	0.71 (0.071)	0.32 (0.01)	0.39 (0.01)
T6	30.61 (1.71)	36.96 (1.77)	31.96 (0.41)	33.99 (0.68)	40.1 (1)	44.9 (1.3)	0.47 (0.005)	0.64 (0.065)	0.35 (0.01)	0.4 (0.02)
T7	40.32 (1.12)	47.04 (1.77)	29.91 (0.23)	30.49 (0.36)	42.43 (1.52)	46.3 (1.3)	0.52 (0.031)	0.62 (0.015)	0.45 (0.02)	0.49 (0.02)
T8	43.68 (1.12)	49.50 (1.46)	33.21 (0.45)	37.11 (0.3)	48.43 (1.52)	52.5 (1.51)	0.66 (0.018)	1.06 (0.013)	0.47 (0.01)	0.56 (0.02)

a)AN: Available nitrogen b) AP: Available phosphorus c)AK: Available potassium d) Org-C: Organic carbon e) T-N: Total nitrogen Values given in table are of 'mean' of five replicates and values in parentheses are of standard deviations

Analysis of soil property after 45 and 90 days of sowing treated with different PGPR formulations shows varying results. In PGPR inoculants treated saline soil available nitrogen was higher in T8 (49.5 mg/kg ±1.46), T7 (47.04 mg/kg ±1.77) and T4 (44.32 mg/kg ±1.27) after 90 days and increase in nitrogen content in T2, T5 and T6 as compare to T3 and T1 (control). Phosphorus content of soil highest in T8(37.11 mg/kg ±0.3) and T6(33.99mg/kg ±0.68) after 90 days and other treatment shows increase in phosphorus content of soil as compare to control. Potassium content of saline soil increases by treatment with PGPR inoculants after 90 days and highest in T8 (52.5mg/kg ±1.51) and other treatments shows increase in potassium content as compare to control. Organic carbon content of soil is an important factor for fertility. In PGPR inoculants treatments organic carbon content was found to be highest in T8 (1.06 %± 0.013%) along with other treatments as compare to control. Total nitrogen content of soil was found to be highest in T8 (0.56 %± 0.02) along with other treatments as compare to T3 and control.

Effect of inoculants on growth of sugarcane in saline soil

Table 6: Effect of PGPR inoculants on germination, shoot length, and shoot fresh weight, total chlorophyll content and total nitrogen content of leaf in sugarcane pot assay

Treatment	Germination (%)	Shoot Length (cm)		Shoot fresh weight (gm.)		T-Chl content (mg/g)		T-N %	
		45days	90days	45days	90days	45days	90days	45days	90days
T1	33.33	56.86 (1.36)	62.74 (1.0)	37.23 (1.7)	40.48 (1.18)	5.19 (1.0)	5.34 (0.97)	0.28 (0.01)	0.26 (0.01)
T2	75	70 (1.45)	100.2 (1.66)	40.22 (1.28)	45.17 (0.99)	10.47 (1.04)	11.27 (1.38)	0.46 (0.01)	0.5 (0.01)
T3	75	67.5 (1.01)	97.54 (1.63)	39.63 (1.06)	42.68 (1.14)	8.41 (1.15)	9.01 (1.23)	0.3 (0.02)	0.29 (0.01)
T4	91.66	72.96 (1.26)	100.2 (1.1)	42.44 (1.18)	47.84 (1.04)	10.67 (1.16)	12.38 (1.29)	0.48 (0.01)	0.51 (0.01)
T5	58.33	70.83 (1.46)	102.1 (1.41)	43.36 (1.16)	46.81 (1.05)	9.97 (1.49)	10.83 (1.14)	0.38 (0.02)	0.41 (0.02)
T6	75	73.16 (1.37)	104.2 (1.0)	44.46 (1.19)	48.07 (1.05)	10.84 (1.28)	12.25 (1.06)	0.38 (0.02)	0.43 (0.02)
T7	83.33	80.13 (1.19)	105.1 (1.16)	46.4 (1.12)	49.25 (1.03)	12.11 (1.33)	14.09 (1.2)	0.51 (0.02)	0.56 (0.01)
T8	91.66	91.33 (1.0)	108.5 (1.27)	50.08 (1.08)	52.28 (1.1)	14.59 (1.44)	16.57 (1.96)	0.57 (0.02)	0.61 (0.01)

values given in table are of 'mean' of five replicates and values in parentheses are of standard deviations

Among the treated pots along with control 91.66 % sugarcane eye bud germination was found to be highest in T4 and T8. In sugarcane plantlets highest shoot length was observed in T8 (108.5 cm ±0.63) and other treatments shows increase in shoot length as compared with control. Sugarcane shoot fresh weight (52.28 g± 1.1) highest in T8 after 90 days and 45 days increase in fresh weight of sugarcane after treatments with other PGPR inoculants as compare with control. The total chlorophyll content of the leaf was estimated and observed that the 16.57 mg/g ± 1.96 in T8 along with other PGPR treatment highest than control. Total nitrogen content of leaf highest in T8 (0.61%±0.01) and increase in other treatments as compare to control.

Application of different formulations of PGPR inoculants in saline soil shows varying results for changing saline soil properties and plant growth promotion. Consortia of *Azotobacter* spp. *Rhizobium* spp. and *Azospirillum* spp. show highest effect than other treatments.

Statistical analysis

The main aim of this study is to check effect of PGPR on saline soil properties and plant growth promotion. To find out the proper combination of PGPR inoculants to relate above statement, we analyze the obtained data for regression. Here, we select electrical conductivity (EC) as soil salinity parameter; Available nitrogen (AN), available phosphorus (AP) and available potassium (AK) are selected as major nutrient content of the soil and shoot length (SL) of sugarcane selected as a plant growth parameter.

Table 7: Regression equations between shoot length and components of soil

Regression equation	R^2
Electrical Conductivity = 3.68- 0.03 T8 AN +0.03 T8 AP -0.01 T8 AK	0.99
Shoot length= 186.73-9.65 T2 pH - 4.81 T2 EC + 0.92 T2 SAR	0.99
Shoot length= 164.33-0.76 T7 AN - 0.81 T7 AP +0.03 T7 AK	0.99

We analyze regression of eight formulations of each of the combinations. The statistical model was developed by applying multiple regression analysis using the obtained experimental data for plant growth, salinity parameters and nutrient content of soil. ANOVA was performed to determine the significant model. This

significant model was determined on the basis of calculated *t*-value and *p*-value. Regression coefficient was determined to predict the accuracy of the model. A 95% confidence interval was used.

Regression of EC with AN, AP and AK

From regression analysis of all data showed that decrease in EC (dependent variable) is highly affected by T-8 AN, T-8AP and T-8 AK (independent variable) of saline soil. There is significant multiple correlation coefficients (0.9998) between decreases in EC of saline soil and increases AN, AP and AK of the soil three variables of nutrient of soil. Available nitrogen, available phosphorus and available potassium 99.9 % of variation decrease in electrical conductivity. It fits multiple regression line for electrical conductivity of soil on available nitrogen, available phosphorus and available potassium of saline soil.

Regression of SL with pH, EC and SAR

Data shows the estimated coefficients of regression analysis of SL (dependent variable) versus pH, EC and SAR (independent variables) of soil treated with PGPR inoculants. There is significant multiple correlation coefficients (0.9999) between increases in SL of sugarcane in saline soil and decrease in pH, EC and SAR of the soil three variables of salinity. pH, EC and SAR contribute 99% of variation increase in SL. It fits multiple regression line for shoot length of sugarcane on pH, electrical conductivity and sodium adsorption ratio of saline soil.

Regression of SL with AN, AP and AN:

From regression analysis of all data it showed that the increase in SL (dependent variable) is affected by increasing in the nutrient content (independent variable) of the treated soil by T-7 AN, T-7 AP and T-7 AK. There is significant multiple correlation coefficients (0.9995) between increases in SL of sugarcane and increases AN, AP and AK of the soil three variables of nutrient content of soil. Available nitrogen, available phosphorus and available potassium 99.95 % of variation increase in shoot length. It fits multiple regression line for shoot length of sugarcane on available nitrogen, available phosphorus and available potassium of saline soil.

From the Table 7 it was observed that for all three regression models R^2 is closed to 99 percent. This indicates that these three regression models have greater ability to estimate the values of shoot length and electrical conductivity accurately.

DISCUSSION

Soil chemical, physical and biological parameters can serve as indicators of treatment effect on soil processes that contribute to nutrient flow in ecosystem. Plant rhizosphere is known to be preferred ecological niche for different types of soil microorganisms due to availability of nutrients, which in turn is intimately related to successful production of crops and sustenance of soil fertility. One of the approaches to explore soil microbe diversity for PGPR having plant growth promoting activities which are well adapted to the particular soil environment [11]. In this context PGPR isolated from saline soil environment are halo tolerant. Efficient uptake of nutrients from soil by roots of plants is a critical issue and rhizosphere isolates can be better competitors due to their direct linkage with roots. Therefore, isolates are added as a bio inoculant or bio fertilizer can improve the nutrient mobilization and improve nutrient status of soils and crops. PGPR inoculants act as nutrient management strategies in various crop including cereals, rice, legumes, wheat [7, 15]. Our halo tolerant PGPR isolates were IAA producer and solubilizes phosphates together with fixation of atmospheric nitrogen by *Azotobacter* spp. and *Azospirillum* spp. This is supported by report of [16] they showed that salt tolerant diazotrophic bacterial isolates produce IAA, solubilizes insoluble phosphates and having nitrogen fixation activity. Bio inoculant promotes growth and productivity of rice and lady finger in saline soil environment.

Several researchers have reported that plant growth promoting rhizobacteria enhanced plant height and productivity by synthesizing phytohormones thereby increasing availability of nutrients or facilitating the uptake of nutrients by plants. The report of [17] shows that content of nitrogen, phosphorus and potassium in soil increased with inoculation of biofertilizers and simultaneous increase in biomass yield of *Stevia*. In our study, available nitrogen, phosphorus and potassium content in saline soil increased with treatment of PGPR inoculants and also there was promotion of sugarcane plant biomass, shoot length, total nitrogen content and chlorophyll content of leaf. *Azotobacter* spp. and *Azospirillum* spp. have nitrogen fixation activity hence available and total nitrogen content in saline soil increases as compare to untreated saline soil. The report by [9] shows that *Rhizobium* spp. inoculation

promotes growth of non-leguminous plants. In this study use of *Rhizobium* spp. inoculants promotes growth of sugarcane. In current study, it was shown that salinity of soil reduces growth of sugarcane but inoculation of halotolerant PGPR inoculants in saline soil ameliorates salt stress and also growth promotion of sugarcane.

CONCLUSION:

The selected PGPR *Azotobacter* spp., *Rhizobium* spp. and *Azospirillum* spp. were halotolerant. This isolate having alkaline phosphatase activity, IAA and exopolysaccharide production. *Azotobacter* spp. and *Azospirillum* spp. have nitrogen fixation activity. Alkaline phosphatase enzyme solubilises insoluble phosphate salts then phosphorus available to plants for growth. Indole acetic acid acts as precursor for growth. Exopolysaccharide production helps in soil aggregation. Nitrogen becomes available to plants.

Experimental observations suggest that root colonizing bacteria that produce phytoharmones may stimulate plant growth and help in nutrient recycling in rhizosphere microcosm and thus microbes can alleviate the effect of salinity in environment. In addition, PGPR might also increase nutrient uptake by plants from soils and thereby reduce need for fertilizers.

In pot assay of sugarcane, although sole application of *Azotobacter* spp. and *Azospirillum* spp. increases available nitrogen, phosphorus and potassium and *Rhizobium* spp. increases available phosphorus and potassium content in soil with sugarcane growth stimulation over control. The results suggested that combined application of bio inoculant (Azt+Rhi+Azo) has been found to be further increases significantly over sole application, in order to derive growth of sugarcane in saline soil. Statistical analysis shows that decreasing in salinity of soil is able to increases in fertility of the soil which promotes growth of sugarcane in saline soil.

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