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Full Length Article

Native Halotolerant Plant Growth Promoting Bacterial Strains can Ameliorate Salinity Stress on Tomato Plants under Field Conditions

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Abstract

Salinity effects plant growth and productivity in many areas of the world. In this research work native halotolerant plant growth promoting bacteria were used to ameliorate salinity stress on tomato plants under greenhouse and field conditions. Isolation of bacterial strains from saline soil was implied as main strategy for better adaptations of bacterial strains under salinity stress conditions. In greenhouse experiment, inoculation of the screened halotolerant bacterial strains increased shoot length of tomato plants ranging between 7.2 and 63.6% and dry biomass ranging between 5.8 and 48.6%, as compared with the control plants grown under varying salinity stress (100 and 200 m*M* NaCl) conditions. Based on greenhouse evaluations, two best performing plant growth promoting halotolerant strains i.e., A12 and A20 were used in field experiments. Field experiments were performed in salinity affected land patches present in agricultural fields of University of the Punjab. Bacteria were provided in the form of sugarcane pressmud based formulations. Both of these strains (A12 and A20) significantly increased shoot length (27.3 and 21.8%) and yield of tomato plants (24.2 and17.3%) respectively grown under natural salinized condition. The strains were identified by16S rRNA gene sequencing as *Bacillus megaterium* strain A12 and *Pseudomonas putida* strain A20. *Bacillus megaterium* strain A12 was used to elucidate mechanisms beneath salinity tolerance in tomato plants based on its superior performance under field conditions. Symbiosis of this strain significantly reduced endogenous ethylene production and increased water use efficacy and production of different enzymes (APX, CAT and SOD) involved in destruction of reactive oxygen species inside tomato plants grown under saline stress conditions. In summary, this study indicates that these halotolerant bacterial strain can be used in conventional agricultural system of Pakistan to rescue growth of plants under salinity stress conditions.

Keywords: Salinity tolerance; Reactive oxygen species; Tomato; Biochemical mechanisms

Introduction

Approximately 1000 million hectors i.e., 7% of the world's land area is salt-affected. In Pakistan, about 6.3 million hectors of land believed to be affected by salinity (Qureshi and Barrett-Lennard, 1998). Pakistan is primarily an agricultural country possessing a wide variety of seasonal, soil and ecological conditions. Total cultivated area of Pakistan is about 79.6 million hectors with vast land resources and only 27% of this area is suitable for cultivation (Ahmed and Qamar, 2004). Along with other abiotic factors, prevailing of soil salinity is major factor limiting agricultural production. Sodium chloride is the most common salt found in saline soils. High level of salinity restricts the crop productivity by influencing different plant growth parameters such as germination of seeds, growth and blooming with lower quality of crops (Ashraf and Foolad, 2007; Arora *et al*., 2008).

Tomato is an important horticultural crop that is used by human in terms of health and nutrition. Tomato is an important constituent of daily diet and source of potassium, vitamins, folic acid and other medicinally important components such as lycopene and β-carotene (Tang *et al*., 2008). Like other crop plants, tomato (*Lycopersicon esculentum* Mill.) growth is negatively influenced by prevalence of salinity in soil (Katerji *et al*., 2003).

The saline land utilization for agricultural purpose is one of the major concern of modern agricultural sciences. Plant growth promoting rhizospheric bacteria (PGPR) is a group of beneficial microbes residing in the vicinity of rhizosphere, colonizing the plant root tissues (Glick, 1995). Crop productivity in saline soils can be increased by the use of different bacterial isolates that colonize the rhizosphere of plants. Various ecological habitats have been explored in order to isolate and characterize the PGPR and these involved rhizosphere of different crops (Hariprasad and

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Niranjana, 2009), saline soil (Upadhyay *et al*., 2009) soil with different contaminations including solid waste of municipality (Krause *et al*., 2003) and cow dung (Swain *et al*., 2008). These microbes have the ability to trigger the growth of plants by means of a vast array of mechanism (Glick, 1995; Glick *et al*., 1998). In this research, native halotolerant bacterial strains were evaluated to ameliorate salinity stress on tomato plants under greenhouse and field conditions.

Methodology

Isolation and Screening of Halo-tolerant Bacterial Strains

Five major districts (Lahore, Faisalabad, Muzaffargarh, Rahim yar Khan and Jhelum) of Punjab province were visited and saline soils samples were collected from salinity effect land patches showing accumulations of salt crusts. Bacterial strains were isolated by standard soil dilution method and colonies were purified by successive transformations on fresh growth media.

Screening of halotolerant bacterial strains was carried out by observing growth of previously isolated bacterial strain on LB broth medium. After incubation of inoculated media at 37°C for 72 h, optical density (OD) at 600 nm for all the bacterial cultures was measured. Strains showing OD >1 were declared as halotolerant and used in further experimentations.

Biochemical Characterization of Halotolerant Bacterial Strains for Presence of Plant Growth Promoting Traits

Selected halotolerant bacterial strains were further screened for presence of biochemical properties positively influencing plant growth. For that purpose, IAA production was assessed by using Salkoviski reagent method and development of pink color in bacterial culture filtrates was observed to ensure IAA production (Benizri *et al*., 1998). Siderophores production was observed by adopting CSA dye methodology of Pérez-Miranda *et al.* (2007). Pikovskaya's agar (Pikovskaya, 1948) medium was used to check phosphate solubilization in terms of clear zone around each colony of bacterial strain. Lastly, ACC deaminase activity was measured by growing selected bacterial strain in tryptic soya agar (TSA) medium containing 0.85 *M* NaCl and 1-aminocyclopropane-1-carboxylic acid (ACC) solution (Penrose and Glick, 2003).

Potential of Selected Bacterial Strains to Ameliorate Salinity Stress Under Greenhouse Conditions

Greenhouse experiment was carried at "Institute of Agricultural Sciences, University of the Punjab", Lahore. Tomato plants were raised in plastic pots of 60 cm diameter containing sterilizes silt loam soil as growth media. Pots were kept in greenhouse under natural light, day/night temperature conditions. After 15 days of emergence, thinning was performed and three tomato plants were left in each pot with nearly equal height.

Salinity stress was artificially developed by adding 100 and 200 m*M* of aqueous sodium chloride solution in growing media of allotted pots. This saline solution was used to irrigate the pots up till field capacity to ensure privilege of salinity stress. Inoculum of selected halo-tolerant bacterial strains in the form of aqueous cell suspension, was prepaid as described by Akram *et al*., (2013) at concentration of $1.0X^{7-8}$ cells/mL. One hundred mL of this inoculum was provided in allotted pots according to experiment design and growth parameters were assessed after 30 days post inoculation (DPI). Control plants were provided with distilled sterilized water for both saline and bacterial treatments.

Molecualr Identification of Selected Halotolerant Bacteria

Selected best performing halotolerant bacteria were identified by 16S rRNA gene sequence study. The bacteria were grown in LB broth medium and the DNA was extracted by using bacterial DNA extraction kit (Enzynomics, Korea) according to provided instructions. The desired gene was amplified by routine PCR using universal forward primer 27F; 50-AGAGTTTGATCCTGGCTCAG-30 and reverse primer 1492R; 50-GGTTACCTTGTTACGACTT-30. The PCR was carried out by using 2X nTaq ready mix "Enzynomics Korea" under temperature conditions as described by Akram *et al.* (2013). The PCR products were sequenced and bacteria were identified by performing BLAST analysis.

Induction of Salinity Tolerance in Tomato Plants in Salt Affected Fields

Field experiments were conducted for two consecutive years for evaluating the effect of selected halo-tolerant bacterial strains in a salt affected field patch (EC; 5.13 dS m^{-1}) at agricultural fields of University of the Punjab, Lahore. Split plots of 2x2 m^2 were prepared containing two raised beds for plants cultivation. Fifteen days old tomato seedlings were transplanted on both sides of raised beds at the rate of 10 seedlings per plot. Sugarcane press mud based formulation of two selected bacterial strains was prepared based on the methodology and applied at the rate of 0.5 kg/plot at the time of transplanting. Same set of field experiment was performed in experimental station of "Institute of Agricultural Sciences, University of the Punjab", Lahore to act as non-saline control. There were three replications plots of each treatment following Randomized complete block design (RCBD). No fertilization or chemical amendment was applied during this field experiment. Growth and yield data were denoted after 60 and 90 days post transplantation respectively.

Elucidation of Physiological Mechanisms Behind Salinity Tolerance in Tomato

The study was concluded by elucidation of possible physiological mechanisms behind amelioration of salinity stress mediated by best performing bacterial strain under field conditions. For that purpose, another independent greenhouse experiment was performed as described in above section. Here only one bacterial strain was used that performed best under filed conditions (*B. megaterium* A12). Afterwards following analysis were performed.

Quantification of Oxidative Stress Related Enzyme

Reactive oxygen species arrest growth of plants under stress conditions. To elucidate mechanism behind amelioration of salinity tolerance in tomato plants under influence of bacterial inducers, some enzymes like CAT APX and SOD were quantified in a time course manner that are involved in degradation of reactive oxygen species.

To extract plant proteins, 100 mg of leaf tissues were crushed in extraction buffer (50 m*M* Tris hydrochloride buffer (pH 7.0) followed by centrifugation at 2500×g for 15 min at 4°C. Upper clear extract was used for enzymes quantifications. Catalase activity was quantified by adopting Aebi (1984) method. Briefly the decomposition of H_2O_2 was monitored by observing change in OD at 240 nm, and 1 U of CAT was defined as mg H_2O_2 released mg^{-1} $protein^{-1}$ minute. APX activity was determined by adopting methodology of Asada (1992). The reaction was started by addition of H_2O_2. The H_2O_2 mediated oxidation of ascorbic acid was noted by taking absorbance at 290 nm. One unit of APX is the amount of enzyme that oxidizes one mole ascorbic acid/min. SOD activity was measured by examining reduction of NBT using the method of (Dhindsa *et al*., 1981). The plant enzyme extract was interacted with 75 m*M* NBT in the presence of 13 m*M* methionine and 2 m*M* riboflavin. The change in OD was read at 560 nm.

Analysis of Change in Ethylene Production

Tomato plants were enclosed in sealed glass bottles and left at room temperature overnight. Five hundred µL sample was taken from neck region of glass bottle and the ethylene contents were measured by Gas Chromatography (GC) according to the temperature conditions as described by Porcel *et al*., (2003).

Statistical Analysis

Data were statistically analyzed by performing analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) using the DSAASTAT package (Onofri Italy).

Results

Isolation of Halo tolerant Bacterial Strains from Soil Samples

In first phase of study bacterial strains from saline soil of different districts of Punjab province. Initially 37 strains were isolated and purified by successive sub culturing (Fig. 1). Further halotolerant bacterial strains were screened by growing them in liquid media amended with 20% NaCl. Eight bacterial strains viz: (A5, A6, A9, A11 A12, A16, A17 and A20) were denoted as halo tolerant by showing OD of >1 at 600 nm (Fig. 1).

Biochemical Characterization of Halotolerant Bacterial Strains for Presence of Plant Growth Promoting Traits

In this experiment selected halotolerant bacterial strains were evaluated for plant growth promotion traits like phosphorous solubillization, IAA production, siderophore production and ACC Deaminase activity (Table 1). Strains like A5, A6, A12 and A20 were found positive for IAA production by presenting pink color on CAS media. Strains A9 and A12 were capable of siderophores production (Table 1). Four strains (A5, A9, A12 and A20) were found positive for phosphorous solubilization when phosphorous was provided in the form of insoluble calcium phosphate. Five strains (A5, A6, A9, A12 and A20) were possessing this trait. Maximum significant activity was observed for strains A12 followed by A20 (Table 1).

Potential of Selected Bacterial Strains to Ameliorate Salinity Stress Under Greenhouse Conditions

Findings of this experiment revealed that these selected halotolerant bacterial strains helped the tomato plants not only in tolerating salinity stress but also positively influenced plant growth and development. Among different halotolerant bacterial strain, maximum significant (ANOVA and DNMRT at p=0.05) growth was induced by strains A12 and A20 under both salinized and normal conditions (Table 2 and Fig. 2).

Plants showed enhanced shoot length in the presence of strains A12 (35.2%) and A20 (29.56%), respectively at 200 m*M* NaCl salinity levelas compared to salinized control plants. In the presence of moderate salinity stress (100 m*M* NaCl), the shoot growth was 41.3 and 33.8% higher under influence of same bacteria, respectively. In the same way, colonization of A12 (59.1%) and A20 (53.8%) significantly increased root length of tomato plants as compared to respective control plants under severe (200 m*M* NaCl) salinity stress (Table 2). For moderate salinity stress (100 m*M*), A12 increased shoot and root length by 55.5 and 65.2% than respective control plants (Table 2).

Table 1: Characterization of growth promoting traits of halotolerant bacterial strains

Bacterial strains	IAA production	Siderophore production	Phosphate solubilization	ACC deaminase activity
A5	+	-	+	1.17+0.08[b]
A6	+	-	-	1.32+0.17[c]
A9	-	+	+	0.36+0.03[e]
A11	-	-	-	-
A12	+	+	+	2.19+0.07[a]
A16	-	-	-	-
A17	-	-	-	-
A20	+	-	+	1.77+0.26[b]

(+) = Activity present, (-) = No activity. IAA= indole acetic acid. [a]ACC deaminase activity = µmol α- ketobutyrate mg protein^{-1}h^{-1}. Values given here are mean ±SD of three replications. Small letters represents level of significance as governed by ANOVA and DNMRT at p=0.05

Table 2: Effect of Halo tolerant bacterial strains on tomato plant growth at different salinity levels

Salinity levels	Bacterial strains	Shoot length (cm)	Root length (cm)	Fresh biomass (g)	Dry biomass (g)	Total chlorophyll (mg/g Fw)
0mM NaCl water	Control	22.32[ef]	14.22[de]	33.41[de]	03.76[d-f]	0.57[d-f]
	A05	28.96[bc]	15.96[b-d]	38.73[c]	04.16[cd]	0.81[c]
	A06	27.31[b-d]	16.35[b-d]	34.25[d]	03.82[de]	0.63
	A09	25.30[bc]	15.72[de]	35.97[d]	03.99[de]	0.75[cd]
	A11	23.32[b-d]	16.19[c-e]	39.12[c]	04.54[b-d]	0.70[c-e]
	A12	36.88[a]	23.84[a]	47.10[a]	05.57[a]	1.13[a]
	A16	24.69[de]	16.57[bc]	38.44[c]	04.62[bc]	0.71[cd]
	A17	25.45[de]	17.10[bc]	39.20[c]	04.21[cd]	0.63[de]
	A20	31.72[b]	22.64[ab]	44.86[b]	05.12[ab]	0.98[ab]
100 mM NaCl	Control	18.32[ef]	09.10[cd]	27.22[de]	02.87[b-d]	0.63[cd]
	A05	19.40[cd]	10.20[cd]	29.60[bc]	02.91[b]	0.49[ef]
	A06	22.61[c]	13.85[bc]	30.32[b]	03.06[ab]	0.51[d-f]
	A09	20.10[cd]	10.92[cd]	28.55[b-d]	02.96[b]	0.62[cd]
	A11	17.50[c-e]	09.55	27.55[de]	02.61[cd]	0.58[de]
	A12	28.45[a]	15.90[ab]	33.50[a]	03.72[a]	0.80[b]
	A16	19.10[b-d]	08.60[c-e]	31.74[ab]	02.88[bc]	0.59 [de]
	A17	20.09[cd]	11.15[c]	28.15[b-d]	02.76[bc]	0.72[bc]
	A20	26.36[ab]	16.88[a]	32.10[a]	03.39[a]	0.92[a]
200 mM NaCl	Control	14.10[de]	06.60[f]	22.40[de]	01.93[ef]	0.55[d]
	A05	15.33[cd]	07.10[de]	26.90[bc]	02.11[de]	0.34[e-g]
	A06	16.78[bc]	08.85[bc]	27.60[b]	02.34[b-d]	0.51[d]
	A09	16.84[b]	07.20[de]	25.01[b-d]	02.09[de]	0.40[e]
	A11	15.93[cd]	09.05[ab]	26.30[b]	02.39[bc]	0.37[ef]
	A12	19.76[a]	10.80[a]	28.70[a]	02.78[a]	0.68[ab]
	A16	14.67[c-e]	08.10[b-d]	23.10[cd]	02.06[d-f]	0.39[ef]
	A17	15.45[cd]	09.95[ab]	25.22[b-d]	02.17[de]	0.66[bc]
	A20	17.78[b]	10.95[a]	29.37[a]	02.50[b]	0.72[a]

Values represented here are mena of three independent replicates. Small letters represents level of significance as governed by ANOVA and DNMRT at p=0.05

Similarly, symbiosis of A12 and A20 increased biomass accumulation and total chlorophyll contents at both salinity stress levels. These strains significantly increased fresh biomass (37.2% for A12 and 33.2% for A20) and dry biomass accumulation (23.6% for A12 and 18.7% for A20), respectively as compared to salinized control plants under sever (200 m*M* NaCl) salinity stress. Finally, total chlorophyll contents were higher in inoculated plants than in the corresponding control plants under both salinized and normal conditions (Table 2).

Molecular Identification of Bacteria

Best performing bacterial strains "viz: A12 and A20" were identified based on 16S rRNA gene homology. These two strains were identified as *Bacillus megaterium* A12 and *Pseudomonas putida* A20. BLAST homology showed that the strain *P. putida* A20 exhibited more than 99% alignment homology with strains BCMU106, AMKP7 and A3 of *P. putida*; while *B. megaterium* A12 showed same levels of homology with strains CMZ19, JIUC-I and DJLZ-9 of same species (Fig. 3).

Induction of Salinity Tolerance in Tomato Plants in Salt Affected Fields

Findings of two consecutive field experiments revealed that symbiosis of A12 and A20 significantly (p=0.05) improved the growth and yield attributes as compared to respective controls under both salinized and normal field conditions. Statistical analysis (ANOVA and DNMRT at p=0.05) showed that plant height, number of fruits and fruit weight were significantly increased in inoculated plants.

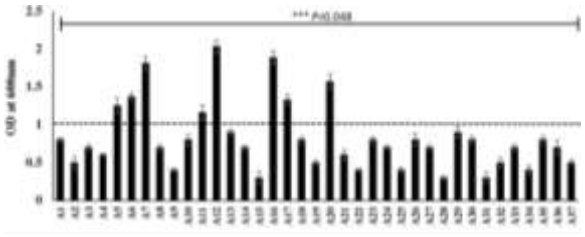

Fig. 1: Population density of halotolerant bacterial strains in growth media amended with 20% NaCl. Vertical bars represent standard error. (***) = Data statiscally significant at $p \geq 0.001$ as goverened by ANOVA

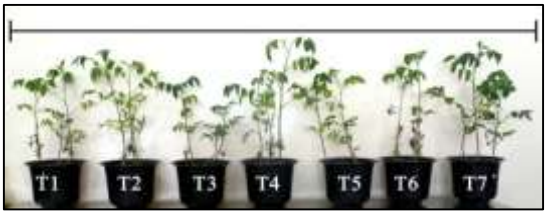

Fig. 2: Effect of selected halotolerant bacterial strains on growth of tomato plants under salinity stress conditions.T1= Plants receiving salinity stress (200 *M*m NaCl) + *P. putida* A20. T2= Plants receiving salinity stress(200 *M*m NaCl) + *B. megaterium* A12. T3= Plants receiving salinity stress (200 *M*m NaCl) alone. T4= Plants receiving salinity stress(100 *M*m NaCl) + *B. megaterium* A12. T5= Plants receiving salinity stress(100 *M*m NaCl) + *P. putida* A20. T6= Plants receiving salinity stress(100 *M*m NaCl) alone. T7= Non-treated control plants

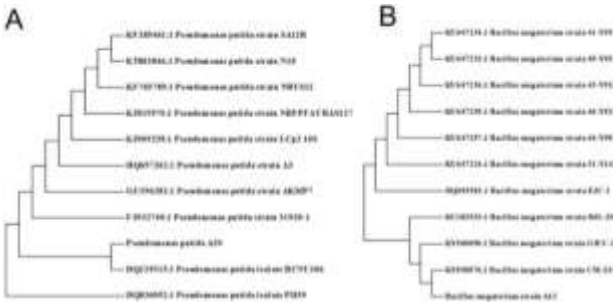

Fig. 3: Maximum likehood method trees based on identification of best performing bacterial strains. A= *Pseudomonas putiba*. B = *Bacillus megaterium*. Phylogentic relationship among 2 bacterial strains isolated from rhizosphere of natural growing plants. Neighbor – joining method was used to infer evolutionary history. The tree was generated using software MEGA 7. Strains names are followed by respective accession number submitted to Gene Bank

Strains A12 and A20 increased shoot lengthby 41.6 and 32.2% as compared to respective control plants in salinityaffected fieldson average basis across both year experiments (Table 3). Moreover, both strains i.e., A12 (24.2%) and A20 (17.3%) enhanced fruit number round both year experiments over the control plant in salinity stress conditions (Table 4). Likewise inoculation of A12 and A20 played effective role in increasing fruit weight of tomato plants as compared to corresponding control plants under all possible conditions.

Elucidation of Physiological Mechanisms Behind Salinity Tolerance in Tomato Plants

Some antioxidant enzymes like CAT APX and SOD were quantified in time course manners in salinized tomato plants under influence of best performing bacterial strain *B. megaterium* A12. The results showed that CAT activity gradually increased in salinized tomato plants. However, in the presence of A12, more pronounced increases were seen as compared to control plants. A robust increase was seen at 2nd day post inoculation (dpi) that started decreasing gradually in successive time points. In the same way, time course study revealed that A12 increased APX activity up to 1.4, 1.7, 3.12 and 2.02 folds at 1, 2, 4 and 8 dpi comparing with salinized control plants (Fig. 4).

Time course analysis depicted that presence of A12 increased SOD levels up to 2.1 and 3.5 folds at 2 and 4 dpi over 0 dpi in the presence of salinity stress. Peak activity was recorded at 4 dpi which started to decrease on later intervals. Like CAT and APX, non-treated control plants maintained consistent lower levels of SOD (Fig. 4).

Analysis of Change in Ethylene Production and Water Use Efficacy

Endogenous ethylene levels and water use efficacy are considered important biomarkers of plants when exposed to abiotic stresses. Endogenous ethylene production was assessed in tomato plants under symbiosis of A12 and salinity stress application. Salinity stress induced significant increased (39%) production of ethylene in tomato plants. Here presence of A12 in rhizosphere of tomato plants significantly decreased ethylene content up to 41.3% as compared to salinized control plants (Fig. 5). Exposure to salinity stress also influenced water use efficacy of tomato plants. Application of A12 significantly improved water use efficacy of tomato plants under salinity stress. Water use efficacy of salinized control plants was 23.9% lesser than the A12 inoculated plants (Table 5).

Discussion

PGPR symbiosis plays an important role in rescuing growth of crop plants during adverse growth conditions. In present study it was found that *B. megaterium* A12 and *Pseudomonas putida* A20 have ameliorative effects on tomato plants grown under salinity stress. Both of these bacterial strains improved the growth of tomato plants under greenhouse and field conditions to maintain its growth during salinity stress.

Table 3: Effect of pressmud based formulations of halotolerant bacterial strains on growth of tomato plants cultivated under saline fields

Treatments	Shoot length (cm)	
	Year 2012	Year 2013
T1	41.17±05.77^{d}	59.82±06.57^{cd}
T2	29.21+03.11^{e}	36.12+04.50^{e}
T3	43.76±06.19^{b-d}	54.98±07.35^{c}
T4	37.29±05.49^{bc}	49.49±04.53^{bc}
T5	69.78±07.53^{a}	71 .80±10.10^{a}
T6	62.23±07.18^{b}	66 .84±09.18^{ab}

Data presented here are mean values of replicates of same treatment. Vales with ± represents standard error. Capital letters represents level of significance as governed by ANOVA and DNMRT at p=0.05. T1= Non-treated control plants. T2= Plants receiving salinity stress alone. T3= Plants receiving *B. megaterium* A12 + salinity stress. T4= Plants receiving *P. putida* A20 + salinity stress. T5= Plants receiving *B. megaterium* A12 alone. T6= Plants receiving *P. putida* A20 alone

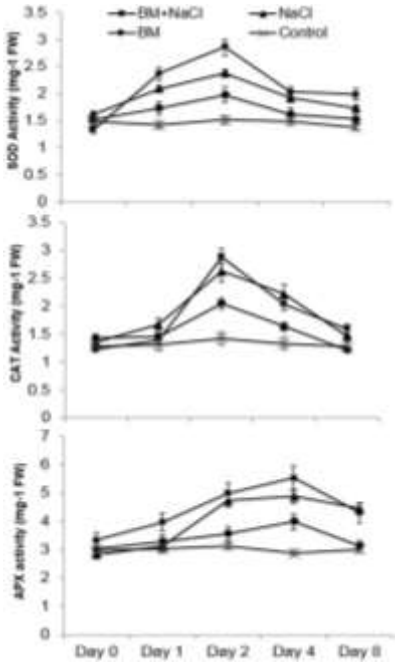

Fig. 4: Change in level of stress related enzymes in tomato plants under influence of salinity and *B. megaterium* A12 (BM) in either combination. Analysis was performed in time course manner. Three technical replicates were made and experiment was repeated twice. Vertical bars represents standard error between replicates of same treatment

Isolation and screening of bacterial strains was done from saline soil samples ensuring better adaptation and performance under salinity. This was implied as main criteria in the present study and saline soil samples were used to isolate bacterial strains. Among all isolated bacterial strains from saline soils, only eight strains (A5, A6, A9, A11, A12, A16, A17, A20) were categorized as halotolerant by sustaining their growth in growth media amended with 20% NaCl. The same method was used by

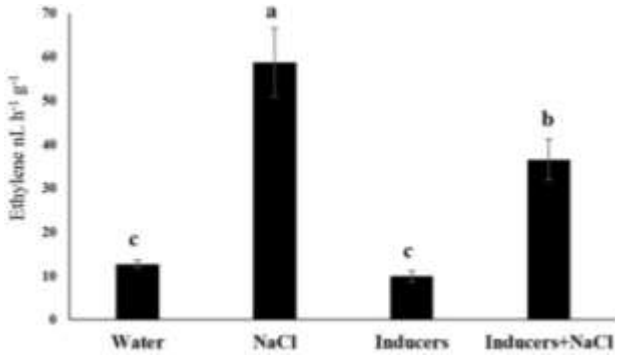

Fig. 5: Analysis of changes in Ethylene levels in tomato plants under influence of *B. megaterium* A12. Data presented here are mean values of replicates of same treatment. Vertical bars represents standard error. Capital letters represents level of significance as governed by ANOVA and DNMRT at p=0.05

Upadhyay *et al*. (2009). Nearly 130 bacterial strains were isolated from salinized soils and 24 were screened as halotolerant that improved plant growth during stress conditions.

The beneficial rhizospheric microbiota relieve plant stress by using different mechanisms (Berg *et al*., 2013; Rolli *et al*., 2015). PGPR increase nutrient acquisition and affect hormones homeostasis (Balloi *et al*., 2010). Some direct mechanisms include auxin and siderophores production, phosphate solubilization and presence of ACC deaminase activity (Glick, 1995; Grover *et al*., 2011). Afterwards, these halotolerant bacterial strains were assessed for presence of aforementioned traits like auxin production, siderophores production, phosphate solubilization and presence of ACC deaminase activity. Our selected best performing halotolerant strains (*B. megaterium* A12 and *Pseudomonas putida* A20) were got positive in most of these traits.

In the next phase of study, *In-plant* experiments were performed and selected halotolerant bacterial strains were interacted with tomato plants in the presence of artificial salinity stress. Our results indicate that some bacterial strains can ameliorate salt stress effectively at greenhouse conditions. Salt stress significantly reduced the growth of tomato plants. However, symbiosis of these microbes significantly increased growth of tomato plants under salinized conditions. The ameliorative effect against salinity stress salt was strain-specific and varying ability was seen for all strains. In this experiment maximum increase in plant growth was provided by two strains "*B. megaterium* A12 and *Pseudomonas putida* A20" at both levels of salinity stress.

There is a dearth of studies demonstration field capabilities of plant growth promoting bacterial strains to ameliorate abiotic stress. It was decided to perform a comprehensive field experiment to demonstrate the ability of our selected two best performing bacterial strains

Table 4: Effect of pressmud based formulations of halotolerant bacterial strains on yield aspects of tomato under field conditions

Treatments	Year 2012		Year 2013	
	No. of fruits/ plant	Fruit weight/5 fruits	No. of fruits/ plant	Fruit weight/5 fruits
T1	19.17±1.96^{b}	819.99±37.83^{b}	23.05±04.04^{ab}	1152.36±31.62^{a}
T2	11.23+02.16^{cd}	312.87±42.15^{e}	08.26±02.14^{e}	427.89±48.69^{d}
T3	16.93+04.11^{bc}	618.72±37.58^{c}	17.42±01.73^{b-d}	813.72±51.20^{c}
T4	15.30±3.87^{c}	584.487±26.14^{d}	19.19±1.93^{bc}	822.28±37.14^{c}
T5	22.07+04.18^{a}	1027.13±97.66^{a}	26.03±03.19^{a}	1342.59±36.72^{ab}
T6	19.15±2.39^{b}	865.64±42.51^{ab}	25.67±2.86^{a}	1197.12±22.52^{a}

Data presented here are mean values of replicates of same treatment. Vales with ± represents standard error. Capital letters represents level of significance as governed by ANOVA and DNMRT at p=0.05. T1= Non-treated control plants. T2= Plants receiving salinity stress alone. T3= Plants receiving *B. megaterium* A12 + salinity stress. T4= Plants receiving *P. putida* A20 + salinity stress. T5= Plants receiving *B. megaterium* A12 alone. T6= Plants receiving *P. putida* A20 alone

Table 5: Change in water use efficacy (WUE) of tomato plants under influence of *B. megaterium* A12

Treatments	WUE (based on dry biomass) mg biomass mg^{-1} H_2O	WUE (based on fresh Biomass) mg biomass mg^{-1} H_2O
Non-treated control	5.96±0.09^{c}	18.36±1.20^{c}
NaCl	5.18±0.18^{cd}	16.50±2.13^{cd}
B. megaterium A12	8.03±0.79^{a}	25.74±1.88^{a}
NaCl+ *B. megaterium* A12	7.21±0.53^{b}	21.42±1.04^{ab}

Data presented here are mean values of replicates of same treatment. Vales with ± represents standard error. Capital letters represents level of significance as governed by ANOVA and DNMRT at p=0.05

"*B. megaterium* A12 and *P. putida* A20". Same type of findings were seen under field conditions. Sugarcane pressmud based formulation of both of these strains effectively ameliorated salinity stress in tomato plants grown under naturally effected land patches present in agriculture fields of University of the Punjab, Pakistan. Same type of stress tolerance was observed by Rakshapal *et al*. (2013). They showed that presence of bacterial strains belonging to Pseudomonas and Bacillus genera to mitigate salinity stress on *Ocimum basilicum* L. These beneficial bacterial strains increased the nutrient uptake ultimately growth of plants. Another PGPR strain *Azospirillum lipoferum* was found to enhance growth of maize plants under water stress conditions (Qudsaia *et al*., 2013).

This study was concluded by detailed elucidation of mechanisms behind stress tolerance in tomato plants under influence of a best performing strain *B. megaterium* A12. Tomato plants were co-cultivated with *B. megaterium* A12 under salinity stress conditions in all possible conditions to get a comprehensive view of possible mechanisms involved in that process.

Salinity stress triggers the production of reactive oxygen species (ROS) causing severe damage to cell structures (Meriga *et al*., 2004; Rasmusson *et al*., 2008). However, a protective mechanism comprising antioxidant enzymes machinery i.e., superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT), is activated under stress conditions. These antioxidant enzymes in turn destroy the ROS entities produced during stress conditions in the cell (Baker and Graham 2002; Murphy, 2009; Mishra and Prakash, 2010). In present study, the activities of three selected enzymes viz: SOD, CAT and APX were analyzed in a time course manner. It was seen that activities of these enzymes were increased in tomato plants under influence of bacterial strain and salinity stress. Presence of *B. megaterium* A12 along with salinity stress induced maximum activities of these enzymes. Robust increases were denoted at initial time intervals that became lower in later intervals. Results of this experiment confirms that bacterial inoculated tomato plants were adapted to saline conditions by eliminating reactive oxygen species through increased SOD, CAT and APX activities.

There exists a correlation between ethylene production and plant growth suppression under adverse conditions (Zapata *et al.*, 2004; Glick, 2005). The logic of this experiment was based on the reason that inoculation of the *B. megaterium* A12, could lower the production of ethylene under salinity stress conditions. This bacterium is further supposed to eliminates the inhibitory effects of ethylene and subsequent increase in plant growth (Penrose and Glick, 2003; Glick, 2005; Hontzeas *et al.*, 2005). Results showed significant decrease in ethylene contents in tomato plants receiving *B. megaterium*A12 in the presence of salinity stress. Plants leaves can osmotically adjust under saline stress by maintaining relative water content of leaves (P´erez-P´erez *et al*., 2007). Increased water use efficacy results in reduced rates of salt accumulation inside plant body (Moya *et al*., 2003). In current study, use of *B. megaterium* A12 significantly increased water use efficacy of tomato plants in the presence or absence of salinity stress.

Conclusion

Present study concludes that native halotolerant bacterial strains possessing plant growth promoting characteristics can be used to mitigate salinity stress in conventional

agriculture system of Pakistan. In future, integration of metabolomics, proteomics and molecular data will be more useful to decipher the mechanism of salt-tolerant bacterial strains to understand the changes that help to bypass the salinity stress in plants.

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