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Improved salt tolerance and growth parameters of groundnut *(Arachis hypogaea* L.) employing Halotolerant *Bacillus* cereus SVSCD1 isolated from Saurashtra Region, Gujarat

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ABSTRACT

Plant growth promoting rhizobacteria (PGPR) are used to augment plant growth and productivity of the plants under abiotic and biotic stress conditions. The present study focuses on analyzing the role of halotolerant rhizospheric bacteria isolated from groundnut plant for enhancing the plant growth under salinity stress. A total of 32 isolates were isolated from rhizospheric soils from Dholara and Junagadh, Saurashtra Region of Gujarat (India). All the isolates showed salt tolerance up to 6% sodium chloride and were screened in vitro for the plant growth promoting traits. Only D3 isolate displayed all PGP activities positive and was selected for further analysis. A substantial increase in all the growth parameters was recorded with PGPR D3 in salt stress conditions using autoclave and non-autoclaved soil. The plant showed a significant increase in root length, shoot length, fresh weight, dry weight, number of leaves and biochemical parameters(Chl a,b) in presence of PGPR D3 under salt stress (100mM). Hence D3 can be used as an alternative to chemical fertilizer and use for the enhancement of plant growth under salt stress.

Key words : *Groundnut, Salt stress, PGPR, Saline soil, PGP traits*

Introduction

Soil salinity is a major problem worldwide in coastal regions dedicated to agriculture. Elevated salinity hinders plant growth and productivity due to unfavorable biotic and abiotic factors (Goswami *et al*., 2014; Singh *et al*., 2008). Mixing zone development at freshwater-seawater interface in coastal regions is increasing at an alarming rate resulting in groundwater salinization and increased electrical conductivity (Lu *et al*., 2009). Furthermore, excessive employment of chemical fertilizer in these areas to improve crop production deteriorates the soil quality (Bharti *et al*., 2016). High salinity generates ionic imbalance, ion toxicity and high oxidative stress in plants. These factors result in nutritional deficiency in plants which limits and deteriorates plant growth (Dodd and Pérez-Alfocea, 2012; Shrivastava and Kumar, 2015).

India is a major crop producer having saline coastal area stretching 7.61 million hectare of which 1.2 million hectares is along the coasts of Gujarat (Singh *et al*., 2007). India is the second largest producer of groundnut (*Arachis hypogaea* L.) after China and about 75% of all its production in Gujarat is limited to Saurashtra-Kutch region (Patel, 1966). The region includesa variety of soils such as clay loam (Junagadh), medium black (Jamnagar, Rajkot

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(Dholara)), light medium and hilly soils (Surendranagar) and coastal alluvial soil (Bhavnagar). Groundnut is an economically important Kharif crop that is used for extraction of oil as well as food. *Arachis hypogaea* is sensitive to salinity (Leidi *et al.*, 1992). It has been reported in literature that groundnutseeds cannot germinate and grow at salinity levels exceeding electrical conductivity 8 $mS.cm^{-1}$. Furthermore, there is very less information available for high salinity tolerance by any of the variety of groundnut (Mungala *et al.*, 2008).

An alternative to this problem is sustainable agriculture by exploiting plant associated beneficial microbes that colonize plant niche improving its growth and productivity (Kloepper *et al.*, 1988). Plant growth promoting rhizobacteria (PGPR) was first reported by Kloepper *et al.* for a group of bacteria which promotes plant growth by facilitating the acquisition of nutrients such as solubilization of phosphate, production of ammonia, chitinase, siderophore, hydrogen cyanide and growth promoter such as indole acetic acid (Kloepper *et al.*, 1988; Kloepper *et al.*, 1980). Employment of plant growth-promoting rhizobacteria (PGPR) is cost effective and easy to employ and has attracted the interest of several research groups worldwide (Jiang *et al.*, 2019). There has been limited reports of employment of PGPR for improving groundnut crop production under saline stress conditions (Jiang *et al.*, 2019).

In the present study, we have screened thirty twonative microbial isolates for PGP traits from Junagadh and Rajkot (Dholara) Saurashtra, Gujarat. Furthermore, the objective of the study isto screen an efficient halotolerant isolate and to analyze the effect of selected PGPR on growth of groundnut plants under salt stress (autoclaved and non-autoclaved) with 100 m Msodium chloride concentration *in vivo* pot experiments. The efficient strains can be employed as an alternate to chemical fertilizer and can improve plant-microbe interaction for improved yield and productivity of groundnut crop.

Materials and Methods

Sample Collection and Isolation of Rhizobacteria

The soil was collected from the crop fields of Dholara (D), District Rajkot and Junagarh, District Junagarh (Ju) from the state of Gujarat. The groundnut plants were carefully uprooted from the soil and only the soil adhering to the rootswas put in autoclaved bags for isolation of rhizobacteria.The soil samples were inoculated on agar plates after serial dilution and bacteria with distinct colony morphology were selected,purified and maintained in double autoclave 20% glycerol at ~20°C for further studies.

Characterization of the Soil Sample

The different physicochemical parameters such as electrical conductivity (EC), pH, available nitrogen (mg/kg), phosphorus (mg/kg), potassium (mg/kg), total organic carbon (%), chloride (mg/kg), sulphate (mg/g), sodium (mg/kg), calcium (mg/kg), magnesium (mg/kg) etc. of the tworhizospheric soil samples were conducted.

Screening of Salt Tolerance

The salt tolerance ability of the bacteria was studied by observing the growth of microorganisms on nutrient agar (NB) medium containing different concentrations of sodium chloride (NaCl) ranging from 2-10% (w/v). Theplates were incubated for 48–72 hoursat 37 °C.

All the biochemical and pot experiments were performed in triplicates and standard error was calculated for each parameter. The PGP traits were analyzed with 2% NaCl concentration.

Screening of Salt Tolerant Rhizobacteria for PGP Traits

Phosphate Solubilization

The phosphate solubilization ability of the bacteria wasanalyzed using Pikovskaya's agar plates incubated for 3-4 days and detecting a clearzone around the colonies (Pikovskaya, 1948). The phosphate solubilizing index(PSI) was calculated using the given formula:

$$PSI = \frac{Colonydiameter(inmm) + Halozonediameter\ (inmm)}{Colonydiameter\ (inmm)}$$

Indole-3-AceticAcid (IAA)

IAA production by different isolates was determined using Salkowski's reagent (Gordon and Weber, 1951). The freshly grown cultures of all the thirty twoisolates were transferred into test tubes containing 5 mL Nutrientbroth (LB) broth supplemented with 100 $mg.mL^{-1}$ L-tryptophan and were incubated at 37°C for 2 days. The broth was then centrifuged for 5 minutes at 10,000 rpm. Superna-

tant was transferred to fresh test tubes and 1 mL supernatant add 2 mL of Salkowski's reagent was added in the tubes. The solutions were gently mixed and were incubated at room temperature for 30 minutes. The development of pink color was recorded spectrophotometrically at 530 nm with uninoculated broth as control. The standard curve was plotted with 5-100 mg.mL^{-1} of IAA (Sigma Aldrich).

Siderophore production

Bacterial isolates were assayed for siderophore production on the Chrome azurol S agar medium (Sigma, Ltd.) described by Schwyn and Neilands (Schwyn and Neilands, 1987). Chrome azurol S agar plates were prepared spot inoculated with the isolates and incubated at 37 °C for 48–72 hours. The development of yellow–orange halo around the growth was considered as positive for siderophore production.

Hydrogen Cyanide production

HCN production was determined by the color change of filter paper (Alström and Burns, 1989; Lorck, 1948). 100 µL of bacteria suspension was inoculated on nutrient agar medium (Merck, Germany) contained 4.4 g L^{-1} glycine. Filter papers were soaked in a reagent solution (sodium carbonate 2% and picric acid 0.5%) and placed in the upper lid of Petri dishes. To prevent volatilization, the plates were sealed with parafilm and incubated at 28 °C for 4 days. One plate without inoculation of bacterium was considered as control. If HCN was produced, yellow filter papers changed to light brown, dark brown and eventually turn into reddish-brown.

Ammonia Production

All bacterial isolates were tested for the qualitative production of ammonia as described by (Cappuccino and Sherman, 1996). Overnight grown bacterial cultures were inoculated in 10 mL peptone broth in test tubes and incubated at 37 ± 2 °C for 48 hours. After incubation, 0.5 mL of Nessler's reagent was added in the tubes. The development of a yellowish-brown color indicated the production of ammonia.

Chitinase Assay

Colloidal chitin was prepared according to the modified method described by (Mathivanan *et al.*, 2014). Ten grams of practical grade crab shell chitin (Sigma Aldrich) was mixed with 150 mL concentrated hydrochloric acid (12 N) under continuous stirring for 2 hours at 4 °C. The suspension was repeatedly mixed with 1 litre water and filtered through filter paper. This step was followed four to five times and the pH of the suspension was adjusted to 7.0 by addition of 5 N NaOH. The colloidal suspension was washed several times with double distilled water for desalting. After desalting, the suspension was centrifuged at 8000 rpm for 10 minutes and the precipitate was collected for further use as colloidal chitin.

Chitinasedetection was performed by inoculating bacterial isolates on plate with 1% chitin supplemented in Nutrient agar. After 5 days of incubation at roomtemperature, the zone of clearance due to chitinhydrolysis was recorded as positive chitinase producingbacteria.

Identification of isolates

Based on the results of PGP traits, the isolate D3 exhibited all traits positive and was selected for further studies. The selected isolate D3 was examined microscopically, morphologically as well as by biochemical methods. The biochemical tests performed were motility test, anaerobic growth, Vogus Proskauer test, citrate utilization, acid and gas production from various sugar like glucose, mannitol, maltose, sucrose, fructose, endospore formation, catalase synthesis, starch hydrolysis, casein hydrolysis, growth at various temperature like 20 °C, 30 °C and 40 °C, 2 to 4% salt tolerance ability according to Bergey's Manual of Systemic Bacteriology (Sneath *et al.*, 1986).

Genomic DNA was isolated from bacterial strain using standard protocol (Russell and Sambrook, 2001). The universal primers 27F(52 - AGAGTTTGATCMTGGCTCAG-32) and 1492R (52-TACGGYTACCTTGTTACGACT-32) were used to amplify 16S gene sequences by PCR. Amplified gene sequences were gelpurified using QI Aquickgel extraction kit (Qiagen, Germany) and sequencing was done by Eurofins Scientific laboratory, Bangalore. Sequence comparison was then performed with obtained 16SrRNA gene sequences against equences in NCBI. The phylogenetic tree was constructed with MEGA version 6 Software (Kumar *et al.*, 2018; Tamura *et al.*, 2004).

Antibiotic Sensitivity Assay

Antibiotic sensitivity or resistance of bacterial strain

was carried out following the method described in the literature (Bauer *et al*., 1966; Jetiyanon *et al*., 2003). The susceptibility of the strains was evaluated by using antibiotic discs of Ampicillin (AMP-20µg), Chloramphenicol (CAF-25 µg), Gentamycin (GM-10µg), Kanamycin (K-30µg), Rifampicin (RIF-15µg), Streptomycin (S-25µg) and Tetracycline (TET-30µg). The isolates were spread on Muller-Hinton agar (MHA) media plates and dried. The antibiotic discs were then placed on MHA plates. The plates were incubated at 37 °C for 24 hours. The zone of inhibition was measured (mm) surrounding the discs of each antibiotic.

Assessment of Antifungal Activity

The agar well diffusion method was used to assess antifungal activity (Mehmood *et al*., 1999). Antifungal activity of bacterial strain was tested against *Aspergillus flavus* and *Aspergillus niger*. The test fungi were grown on potato dextrose agar (PDA) and the fungal spores were suspended in sterile normal saline. Spore suspension (0.1 mL) was spread on fresh PDA plates and wells of 8 mm diameter were punched into the agar medium and filled with 100 µL of bacterial culture. The plates were incubated for 5-6 days at 27 ± 2 °C.

Pot Experiment

The soil employed in pot experiments was having pH 8 and electrical conductivity (EC)1.3mS.cm^{-1}. Groundnut seedswere obtained from the local market of Rajkot, Gujarat. The seeds were further surface sterilized by soaking in 70% ethanol for 1 min followed by incubation in 1% sodium hypochlorite for 3 min followed by 4-5 times rinsing with sterilized distilled water. 1 mL of 24 hour old bacterial culture 10^9 CFU/mL was added on seeds and kept on moist filter paper sheets in the petri dish by adding 10mL autoclaved water. The petri dish was covered with other sterilized filter paper sheets and seeds were allowed for germination. Control seeds were without bacterial culture.This set was incubated for five days (Pongdet Piromyou *et al.*, 2011). The untreated seeds act as control.

For pot trials, the selected seedlings were sownin plastic pots filled with 3 kg of soil. Experiments were triplicated (3 pots per treatment; 4 seed per pot). The treatments were as follows: as control - without salt, (*Control-Salt*) and with salt (*Control+Salt*), PGPR D3 (*D3-Salt*) and PGPR D3 (*D3+Salt*). All the work was done both with autoclaved and non-autoclaved soil. Bacterial culture was directly applied to each of the seedling base two days after plantation of germinated seeds. Pots were watered with distilled water daily for seven days after plantation. Post acclimatization saline waterwas used to induce stress (100 mMNaCl) to maintain salinity in the soil till plant harvesting (30 days). Non-saline plants were irrigatedonly with water.

Growth and Physiological Parameters

The harvested plants were studied for physical growth parameters such as their root height (cm), shoot height (cm), fresh weight, dry weight, and leaf area. Furthermore, biochemical estimations of essential growth parameters were performed such as photosynthetic pigments, and proline content (Bharti *et al*., 2016).

The height of shoot was measured at the time of harvest and root length was measured after uprooting of the plants and rinsing them with water to remove soil. Fresh weight measured of harvested plants followed by their dry weight measurement after drying at 70 °C for 3 days.

Leaf from each plantlet was sampled rinsed with distilled water and dried with filter paper before analysis for leaf area. The total leaf area was calculated by using Kemp's formula (Kemp, 1960):

$$Total\ leaf\ area = l \times b \times K$$

where, l is the length of leaf, b is the breadth of leaf and K is 0.66 (Kemp's constant for Dicot).

For Chlorophyll content determination fresh leaf sample (0.1 g) was homogenized in 80% acetone and filtered through muslin cloth. The filtrate was collected in fresh tubes and the final volume was maintained at 10 mL. The pigments were extracted and the absorbance was measured at 645 nm and 663 nm employing UV-Vis spectrophotometer (Shimadzu, 1100). The quantification of the pigments was done following (Bharti *et al*., 2016; Dashti *et al*., 1997; Porra *et al.*, 1989):

$$Chlorophylla = [12.7(D_{663}) - 2.69(D_{645})] \times [\frac{v}{1000} \times w]$$

$$Chlorophyllb = [22.9(D_{645}) - 4.68(D_{663})] \times [\frac{v}{1000} \times w]$$

where, D_{663} and D_{645} are absorbance at respective wavelengths, v is the volume of filtrate (10 mL) and w is fresh weight of leaf (0.1g).

Proline content was estimated by employing nin-

hydrin as described by Bates *et al*., 1973. Plant tissue of 100 mg was homogenized in 1.2 mL of aqueous sulfosalicylic acid (3%) and centrifuged 10000 g for 15 minutes. 500µL of supernatant was collected in fresh autoclaved Eppendorf tubes and 500 µL of distilled water was added and gently mixed. 1 mL of ninhydrin (2% in acetone) was added to the tubes and incubated at 100 °C for one hour. The temperature of the tubes was lowered by incubating them in ice bath for 15 minutes. 2 mL of toluene was added to each tube and vortexed for 3-4 minutes. The upper phase was collected and spectrophotometrically absorbance at 520 nm was recorded. The quantitative estimation of proline was done using standard curve plotted with different concentrations (10-100µg/mL) of L-Proline (Sigma Aldrich, USA).

Results

Isolation and NaCl Tolerance of Rhizobacteria

A total of thirty twoisolates were collected from the rhizosphericsoils of groundnut plants grown in the Dholara and Junagarh ecosystem of Saurashtra, Gujarat, India. These isolates were screenedfor their salt tolerance abilities at different concentrations of sodium chloride (2-10% w/v). All thirty two isolates displayed healthy growth in 2-6% NaCl (w/v).Twelve isolates sustained growth even in thepresence of up to10% NaCl (w/v) and 2% NaCl (w/v) were selected for the screening of PGP traits for all the isolates.

Characterization of the Soil Sample

The physicochemical assessment of soil samples was performed from Dholara and Junagadh area of Gujarat. The pH and electrical conductivity were observed as 8.5 and 3.0 mS cm^{-1} for Dholara soil sample whereas for Junagadh soil sample pH 8 and 2.3 mS cm^{-1} was recorded. Table 1 shows the results of the chemical assessment of both the soil samples. Chloride estimation was done by Argentometric titration method (Kolthoff and Kuroda, 1951). Quantification of sodium, potassium, and calcium wasperformed using Flame Photometer (Model: Micro-controller based with Compressor, Type: 128 Systronics). Estimation of phosphate and sulphate was conducted using UV-Vis spectrophotometer (Shimadzu UV-1900). Magnesium was estimated using Atomic Absorption Spectrophotometer (Perkin Elmer, Model AANALYST 400). Total nitrogen estimation was done using KJELO PLUS Automatic Nitrogen/Protein Estimation (ISO 9001:2008 Certified & CE Certified). The total organic carbon was estimated using Walkley-Black titration method (Storer, 1984).

Sodium and nitrogen content were observed to be 85% and 63% higher in soil from Dholara as compared to Junagadh. Junagadh soil sample had higher concentrations of calcium and sulphate (8% and 78% respectively). Less than 1% change was obtained in available phosphorous, chloride, and total organic carbon. There is an increase in potassium content by 7.4% inDholara than Junagadh. Thus the soil of Dholara was fertile with respect to macronutrients than Junagadh. The physicochemical characters of the soil and environmental factors greatly influence the types and growth of microorganisms in plant proximities.

Screening of Salt Tolerant Rhizobacteria for PGP Traits

Microbial isolates enables plant growth promotion by facilitating the production of indole acetic acid, siderophore, ammonia, hydrogen cyanide, chitinase and phosphate solubilization. All the thirty two isolates were evaluated for PGP traits (Fig. 1a-e). The solubilization of precipitate forms of phosphorous enables easy uptake by the plants (Rodrýìguez and Fraga, 1999).

All thirty two isolates displayed phosphate solubilization, which was indicated as clear zones on Pikovskaya's agar plates (Fig 1a). Plant growth promoter, indole acetic acid was observed to be pro-

Table 1. Assessment of chemical properties of soil sample collected from the rhizosphere of the groundnut plant from Dholara and Junagarh (Tests were performed at Gujarat Institute of Desert Ecology, Gujarat).

	Available nitrogen (mg/kg)	Available Phosphorus (mg/kg)	Potassium (mg/kg)	Total organic carbon (%)	Chloride (mg/kg)	Sulphate (mg/g)	Sodium (mg/kg)	Calcium (mg/kg)	Magnesium (mg/kg)
Dholara	257.1	13.4	940	0.42	654.4	0.49	354.5	11080	1656
Junagadh	94	13.3	870	0.4275	654.4	2.24	49.75	12080	864

duced by thirty one isolates in the range of 0-47 µg.ml^{-1} (Fig. 1b). Production of chitinase as a potential antifungal agent helps plants by inhibiting the growth of pathogenic microorganisms in its rhizosphere (Whipps, 2001). Figure 1c shows five isolates-D3, D7, D15, Ju8 and Ju12, displayed chitinase production by obtaining significant zone of chitin degradation on a chitin agar plate. Siderophore facilitates iron, which is a cofactor for nitrogenase enzyme essential for atmospheric nitrogen fixation by plants. None of the isolates from Junagadh displayed siderophore production. Five isolates from Dholara-D1, D2, D3, D4, and D9 displayed siderophore production. Figure 1e indicates nitrogen fixation, ammonia and hydrogen cyanide production by the isolates. All thirty two isolates were tested positive for nitrogen fixation, which facilitates the enhanced amount of available nitrogen in the form of ammonia, nitrates or nitrites in the rhizosphere.

All isolates from Junagadh and only four from Dholara-D3, D14, D15 and D16 displayed ammonia

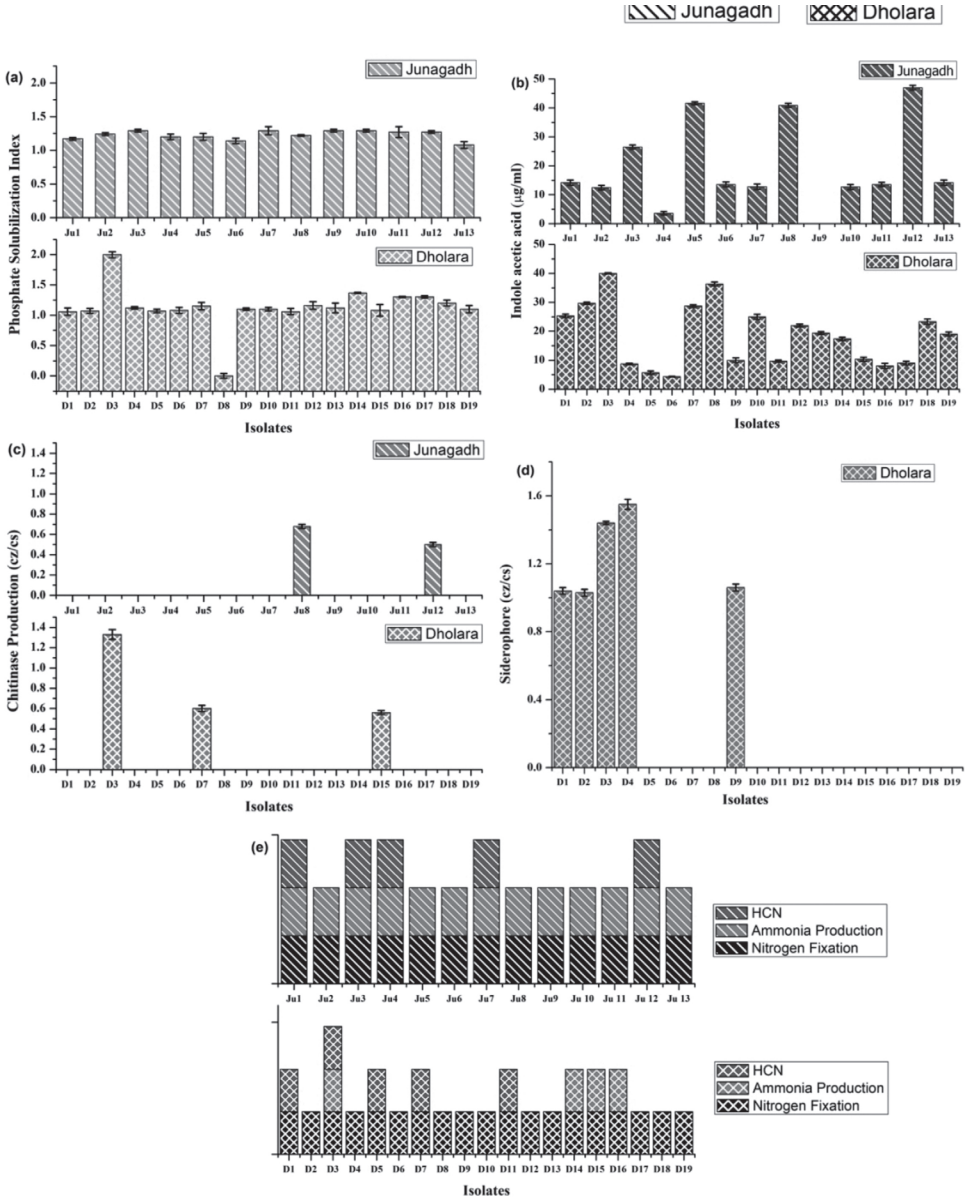

Fig. 1. PGPR traits of thirty two isolates (Junagadh-Ju1 to Ju13) and (Dholara – D1 to D19) for (a) phosphate solubilization index, (b) IAA production µg/mL, (c) chitinase production, (d) siderophore production (cz/cs), (e) nitrogen fixation, ammonia and hydrogen cyanide (HCN) production.

production which directly supports plant growth as it acts like a macronutrient. Ten isolates could produce HCN that suppresses the effects of biotic stresses induced to the plants (Saharan and Nehra, 2011). Furthermore, only one isolate-D3 from all the thirty two isolates was tested positive for all seven PGP traits and was employed for further studies.

Bacterial Identification

The biochemical and molecular characterization of D3 was performed for its identification. Biochemical characterization including Gram reaction, VP test, acid production-glucose, maltose, sucrose, fructose, endospore staining, citrate utilization, and casein hydrolysis showed positive results with D3. D3 was tested negative for catalase test, starch hydrolysis, acid production-mannitol, and anaerobic growth. The bacterial isolate was identified by 16S rDNA sequencing andby microbial identification system. The DNA was extracted and used as a DNA template in PCR reactions. 16S rRNA gene was amplified by using the universal primers pair 8F: AGA GTT TGA TCC TGG CTC AG and U1492R: GGT TAC CTT GTT ACG ACTT. The amplified product obtained in this study was deposited in the GenBank nucleotide sequence database under the accession number *Bacillus cereus* SVSCD1. The 16S rRNA gene along with their closest homology sequences were aligned and implemented in MEGA 6 software.The phylogenetic tree was constructed by neighbour-joining (NJ) method using MEGA 6 program.

The antibiotic profiling of D3 showed sensitivity with various antibiotics. D3 showed a maximum zone of clearance (27.33 mm) with streptomycin and least with rifampicin (17 mm).

D3 was assessed for its antifungal potential with *Aspergillus flavus* and *Aspergillus niger*. Minor zone of inhibitions were observed with both *Aspergillus* species on PDA plate indicating it to be beneficial for plant growth as can inhibit disease causing fungi.

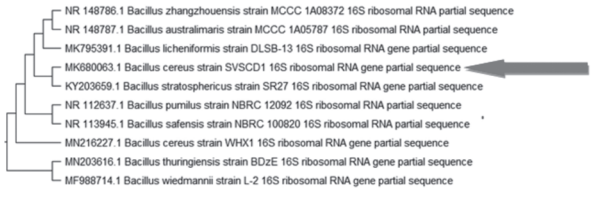

Fig. 2. NJ-phylogenetic tree showing the evolutionary relationship between SVSCD1, a PGPR isolate and reference strains from GenBank database.

Pot Experiment

Plant growth promoting ability of D3 isolate was determined in vivo using pot experiments employing non-saline and saline soil as well as autoclaved and non-autoclaved soil. Chemical properties of soil used for pot study was determined.

The major nutrients- nitrogen, phosphorous and potassium were obtained to be 0.056%, 19.1 $kg.ha^{-1}$, and 167.5 $mg.kg^{-1}$ respectively. The other essential nutrients including calcium, magnesium, sodium, chloride, sulphate and organic carbon were found to be 2961.8 $mg.kg^{-1}$, 94.28 $mg.kg^{-1}$, 210.7 $mg.kg^{-1}$, 42.12 $mg.kg^{-1}$, 10.6 $mg.kg^{-1}$ and 0.404% respectively. The soil quality analysis indicates medium fertility of the soil (Basu, 2011). Furthermore, the soil salinity and electrical conductivity was estimated to be 0.08% and 1.3 $mS.cm^{-1}$ respectively.

The inset of Figure 3 shows the pictures of groundnut plants under control and salt stress conditions with PGPR D3. The shoot length of the plants was observed to be enhanced by 12.3% without salt and 12.06% with salt stress (100 mM) with PGPR D3 using autoclaved soil (Fig. 3a).

However, under non-autoclaved conditions, it was observed to be increased by 19.8% without salt and 29.69 % with salt stress (Fig. 3b). Similarly, an increase in root length was observed with D3 in autoclaved and non-autoclaved soil (Fig. 3c,d).

The variation in biomass was calculated by analyzing the changes in fresh and dry weight. Fresh weight was observed to be enhanced by 18.12% without salt and 11.02% with salt stress (100 mM) with PGPR D3 using autoclaved soil (Fig. 4a). However, under non-autoclaved conditions, it was observed to be increased by 25% without salt and 13.8% with salt stress (Fig. 4b). The dry weight has an enhancement of 16.6% when compared to control without salt in presence of PGPR, while an increase of 20% was obtained with salt concentration under sterilized soil conditions with the isolate. Similarly, an increment of 24% and 25% was observed with and without salt stress with PGPR in non-sterile soil conditions (Fig. 4c,d).

The other physical parameters such as the number of stems and leaves were also evaluated. There is change in the number of stem by one under salt stress with PGPR D3 in autoclaved soil condition (Fig. 5a) and non-autoclaved soil under NaCl stress (Fig. 5b). The number of leaves displayed a substan-

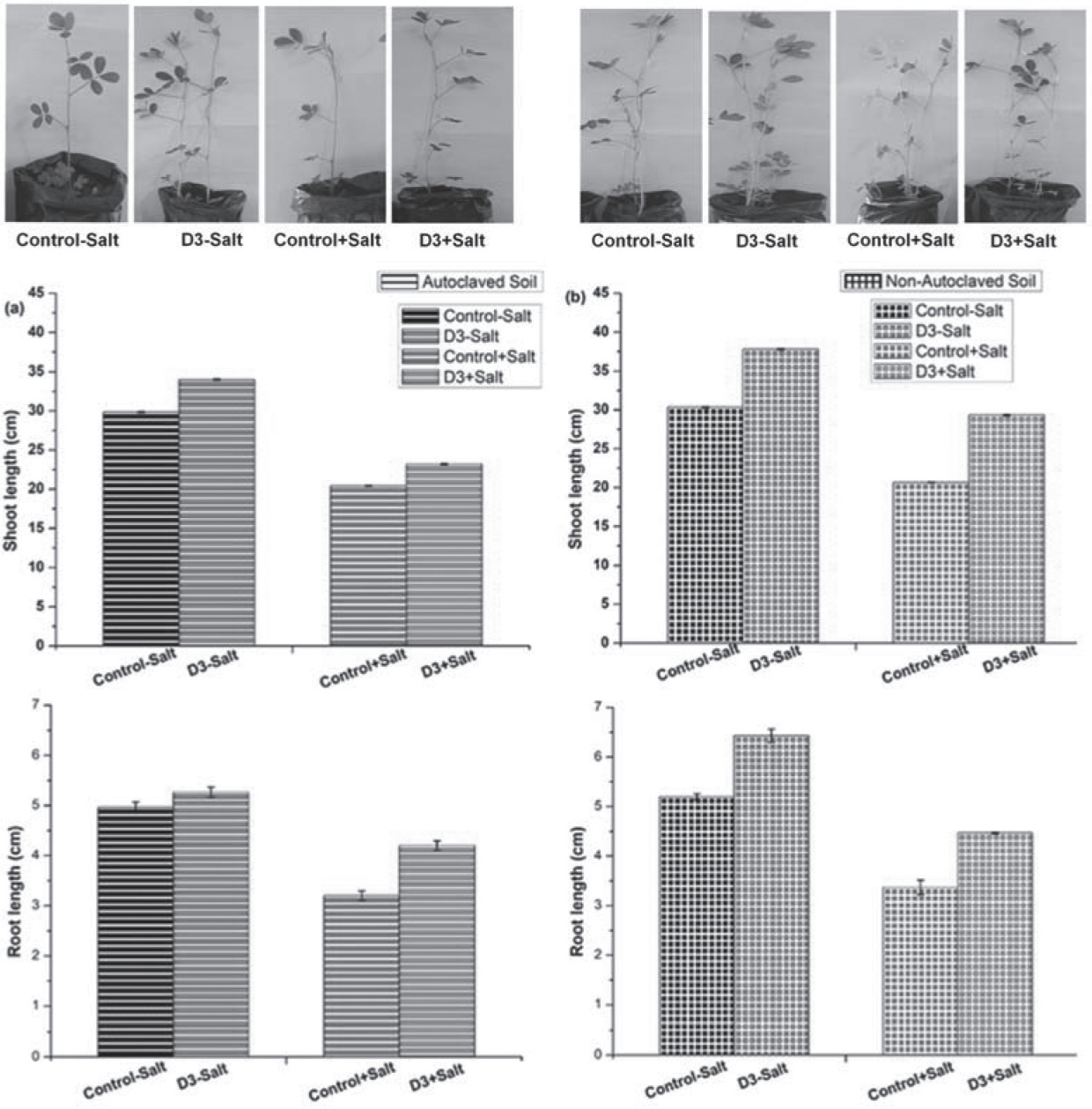

Fig. 3. Effect of PGPR *Bacillus cereus* SVSCD1 in presence and absence (control) of salt stress on (a) shoot length in autoclaved soil, (b) shootlength in non-autoclaved soil, (c) root length in autoclaved soil, (d) root length in non-autoclaved soil. Inset: images of groundnut plants with control. Vertical bars represent the mean ± SEM.

tial increment with 16% and 25% under salt stress with autoclaved and non-autoclaved conditions with PGPR respectively (Fig. 5c,d). The leaf surface area was also observed to be enhanced by 21% and 10% under salt stress in autoclaved and non-autoclaved soil respectively. The study of physical parameters evidently illustrates that PGPR-D3 enhances the growth of groundnut plants under both saline and non-saline soil conditions (Fig. 5e, f).

Furthermore, the quantification of photosynthetic content was performed by estimating chlorophyll a and b (Chl a and Chl b). Concentration of Chl a showed significant enhancement of 30% without salt along with PGPR in autoclaved soil (Fig. 6a).

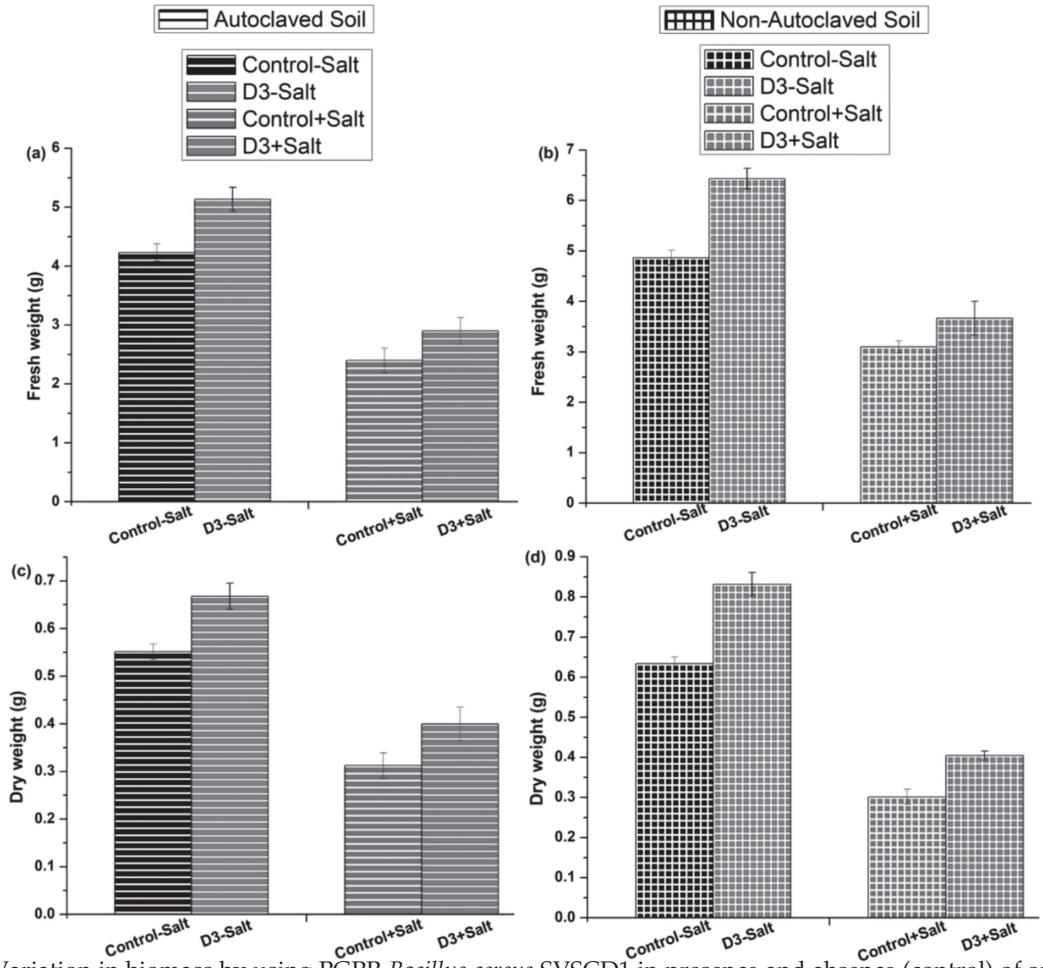

Fig. 4. Variation in biomass by using PGPR *Bacillus cereus* SVSCD1 in presence and absence (control) of salt stress on (a) fresh weight in autoclaved soil, (b) fresh weight in non-autoclaved soil, (c) dry weight in autoclaved soil, and (d) dry weight in non-autoclaved soil. Vertical bars represent the mean ± SEM.

While an increase of 21% with salt stress (100 mM) with PGPR D3 under non-autoclaved soil conditions was obtained (Fig. 6b).

Similarly, for chlorophyll b, an increase of 44% and 55% was observed in the absence and presence of salt stress with PGPR D3 in autoclaved soil, respectively. The significant results were observed with non-autoclaved soil too (Fig. 6c,d) .

Moreover, proline as a stress indicator has no significant increase in under non-saline stress conditions. However, an increase of 20% was obtained for salt stress conditions with autoclaved soil (fig. 6e). Similarly, with non-autoclaved soil conditions, no significant change was observed without salt and 22% increase was obtained with PGPR D3 under saline conditions (Fig. 6f).

Discussion

The enhancement of salinity in the soil is responsible to deteriorate growth and nutrient uptake by the crops (Marschner, 1995; Upadhyay *et al.*, 2012). Salt stress can induce nutrient imbalances in plants that result in inhibited growth (Greenway and Munns, 1980; Yue *et al.*, 2007). In the present study, it was evident that the growth of plant was inhibited and affected when inducted with 100 mM salt stress. There was an evident increment in the growth of the groundnut plants after supplementation of halotolerant PGPR D3 which was screened and isolated from Rajkot, Gujarat.

Several research groups have reported that PGPR may benefit plant nutrition through different mechanisms (Bharti *et al.*, 2016; Kloepper, 1978;

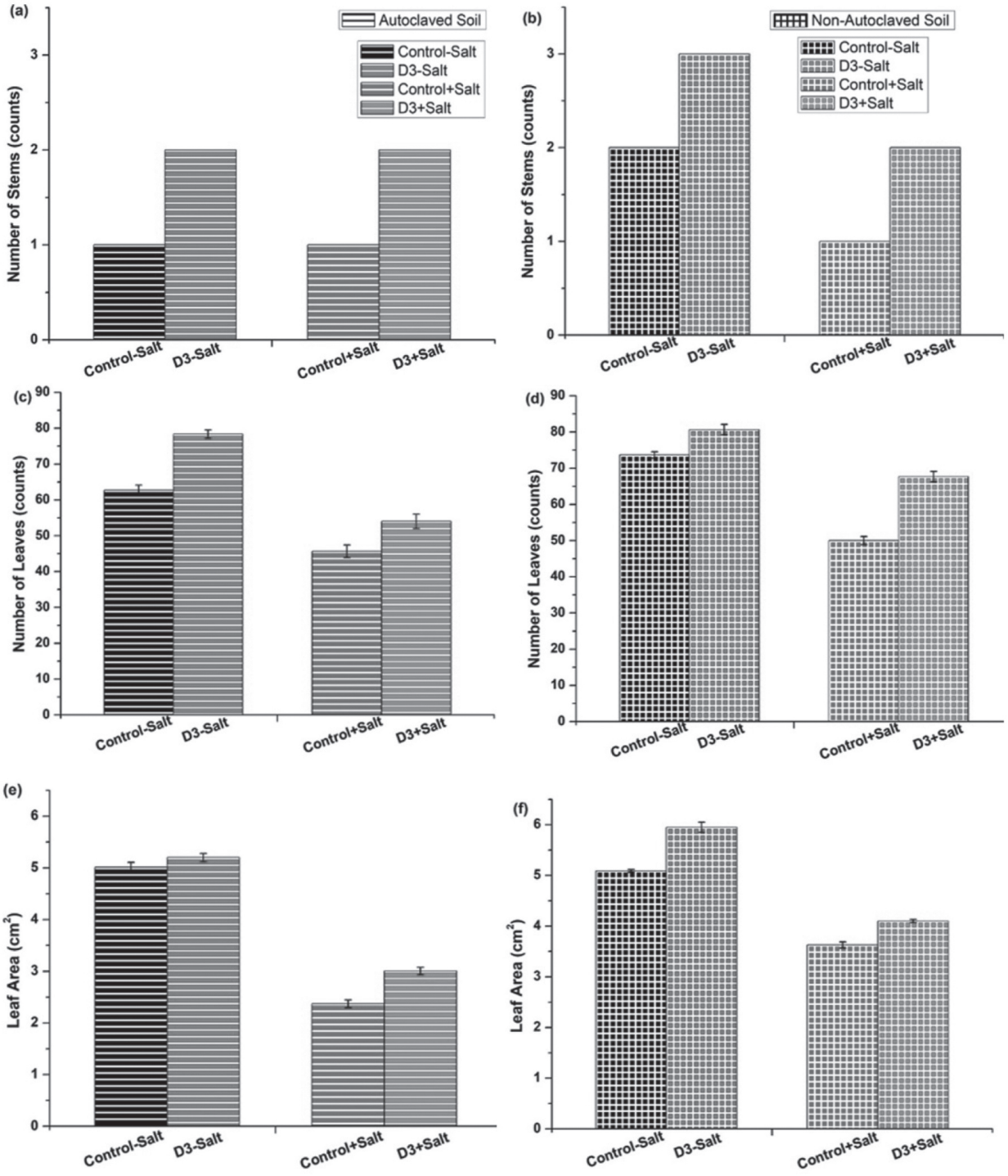

Fig. 5. Effect of PGPR *Bacillus cereus* SVSCD1 in absence and presence (control) of NaCl (100mM) salt stress on(a) and (b) number of stems; (c) and (d) number of leaves; (e) and (f) leaf area with autoclaved and non-autoclaved soil.Vertical bars represent the mean ± SEM.

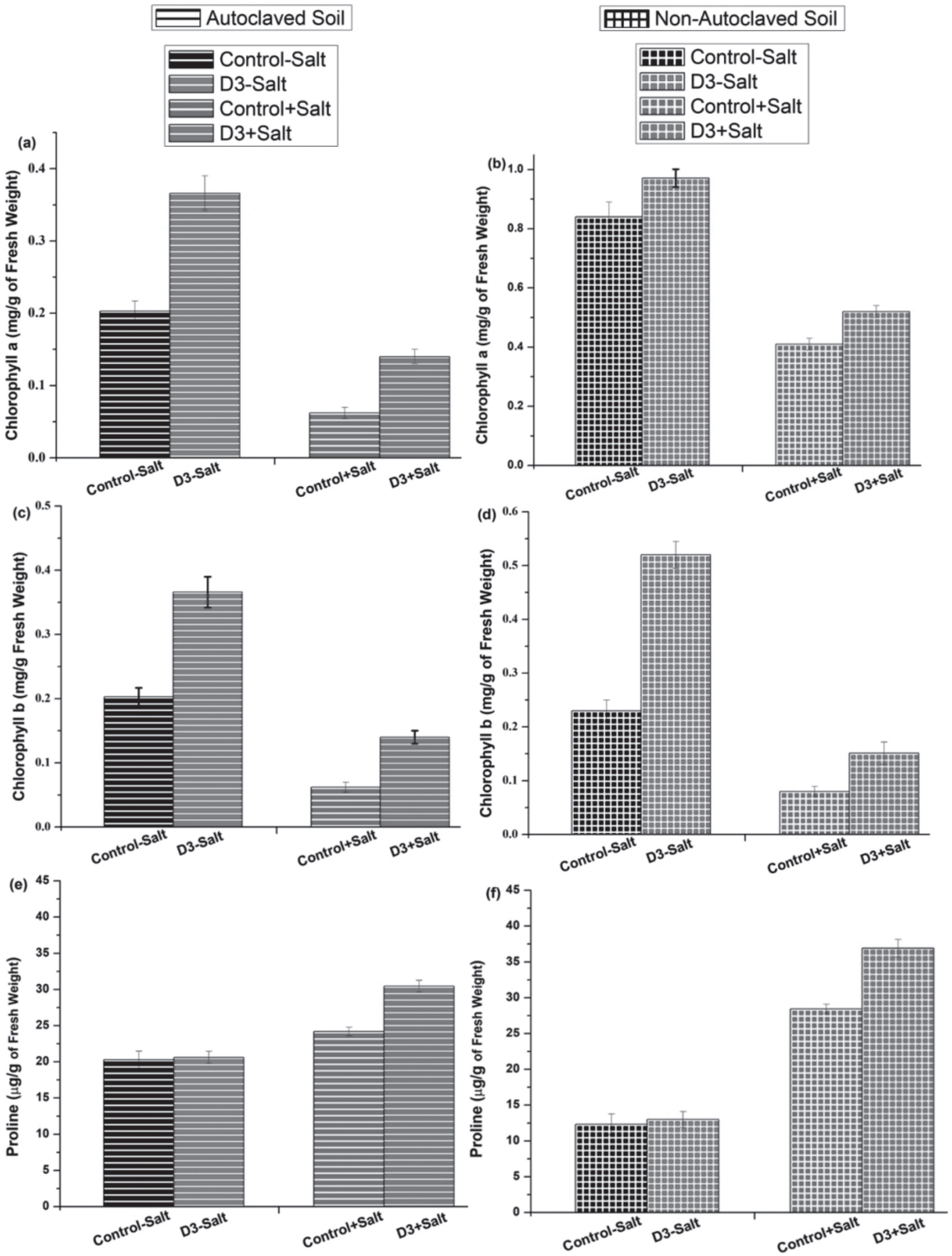

Fig. 6. The changes in biochemical parameters of groundnut by *Bacillus cereus* SVSCD in absence and presence (control) of NaCl (100mM) salt stress on (a) and (b) chlorophyll a (Chl a); (c) and (d) chlorophyll b (Chl b); (e) and (f) proline content with autoclaved and non-autoclaved soil. Vertical bars represent the mean ± SEM.

Upadhyay *et al.*, 2012). Phosphate is essential for all plants for respiration and photosynthesis (Navarro *et al.*, 2001). Salinity affects the uptake of phosphate uptake by plants and hence displays retarded growth. The rhizobacteria in the proximity of root surface is much higher as compared to the bulk soil and utilizes various sugars present in root to produce organic acid. This enables solubilization of phosphate and hence, feasible availability for uptake by the plants. In the present study, all thirty two isolates exhibited phosphate solubilization and phosphate solubilization index was in the range of 1 to 1.37 (Upadhyay *et al.*, 2012). The ability of the strain to facilitate phosphate nutrition to the plant might assist the ability of PGPR-treated plants to overcome salinity stress (Kucey *et al*., 1989; Upadhyay *et al.,* 2012).

The phytohormone indoleacetic acid (IAA) is vital for several essential parameters in the plant including stimulation of root development by increasing the number of root tips and surface area of roots for supplementing nutrition uptake (Kloepper, 1978; Yang *et al*., 2009). The increased bifurcation of roots also can be associated with increased biomass (fresh and dry weight) in plants treated with PGPR (Siddikee *et al*., 2010). Furthermore, it has been reported that PGPR increases plant uptake of essential ions by stimulating the proton pump ATPase. The thirty one isolates produced IAA in the range of 3.6-47 $\mu g.mL^{-1}$, with PGPR-D3 (*Bacillus cereus* strain SVSCD1) producing 40 $\mu g.mL^{-1}$. Furthermore, it has been reported that IAA improved the adverse effects due to enhanced salinity (Kaya *et al.*, 2013).

The transformation of organic nitrogen to ammonia by PGPR is another crucial plant growth promoting trait as it increments the nitrogen content in soil (Dey *et al*., 2004). Moreover, the soilwith higher amounts of nitrogen can accumulate ammonia generating alkaline condition of the soil. The increased basicity can control fungal growth in the rhizosphere (Singh and Jha, 2017). Ammonia production was demonstrated by all thirty two isolates.

Sequestration of iron by siderophore causes iron deficiency on growing pathogens in the plant rhizosphere, and hence indirectly suppressing the growthof these pathogens. Furthermore, several reports suggest that microbial siderophore facilitates plant growth and is an essential parameter for screening of PGPR (Sadeghi *et al.*, 2012). Jan and coworkers have demonstrated the importance of siderophore production in species of *Pseudomonas*. Siderophore producing isolates induced resistance of gummy stem rot in watermelon, while siderophore negative isolates were observed to be infected. 5 isolates from Dholara and none of the isolate from Junagadh was observed with siderophore production. Furthermore, several research reports suggest chitinase and hydrogen cyanide production amongst beneficial traits expressed by PGPR. Chitinase dissociates chitin which is a component of fungal cell wall hence inhibiting their growth in the rhizosphere (Goswami *et al.*, 2014). 5 isolates from thirty two isolates displayed chitinase production. Hydrogen cyanide has been suggested to be a bacterial secondary metabolite. HCN is a desired quality for PGPR as it inhibits the development of plant diseases by enhancing host disease resistance and acts as weedicide (Castric, 1975; Schippers *et al.,* 1990).

Out of all isolates, the selected *Bacillus cereus* strain SVSCD1 was employed for pot assays. There was a significant increment in the groundnut plant growth parameters in the presence of *Bacillus cereus* strain SVSCD1 under salt stress compared to un-inoculated plants using autoclaved and non-autoclaved soil. As the selected strain from the present study exhibited all PGP traits to be positive and promoted the growth of plants in salt stress conditions. So this strain can be employed and exploited as a potential biofertilizer and biofungicide.

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