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Soybean Growth-promotion by *Pseudomonas* sp. Strain VS1 under Salt Stress

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Abstract: In the present study, we employ *Pseudomonas* sp. strain VS1 showed *in vitro* plant growth-promotion characteristics and promoted soybean seed emergence under salt stress. Strain produced indole 3-acetic acid in the presence of salt stresses that exhibited high numbers of lateral root as compared to control. Bacterial strain exhibited growth in DF salt medium amended with 1-aminocyclopropane-1-carboxylate through ACC deaminase activity. Bacterial-treated soybean seeds were subjected to salt stress and significantly enhanced emergence at 7 days after seeding. Strain untreated soybean plants had a 33% seed germination when 200 mM NaCl was applied at 0 DAS and the root length was significantly decreased compared to the strain treated plants (LSD_{0.05} = 0.21). Most importantly, the application of 200 mM NaCl at 0 DAS resulted in only a 9% of lateral root in untreated plants as compared to strain treated plants.

Key words: Plant-microbe interaction, stresses, abiotic stress, IAA production, ACC deaminase

INTRODUCTION

In present scenario plant-microbe interaction is an important and unavoidable approach to explore that result in the promotion of plant health and may apply for improved productivity of Agro-ecosystem. Plants somehow face biotic and abiotic stress which is responsible for low productivity of agricultural crops. Now-a-days researchers are employing stress tolerating microbes that alleviate stresses whereby plant may survive under such conditions. Based on different plant growth-promoting properties, viz., indole-3-acetic acid production (IAA), inorganic P-solubilization and 1-aminocyclopropane-1-carboxylic acid (ACC) we may employ beneficial microbes for cultivation of plants under stress. Choudhary *et al.* (2011) elaborately described role of microbes in alleviation of stress in form of biotechnological perspectives together with mechanistic approaches (Choudhary, 2012).

Abiotic stress, e.g., salt and drought profoundly affects plant growth and reduces production up to the appropriate level. Plant growth-promoting bacteria that grow in presence of high salt and drought conditions may alleviate such stress. Barassi *et al.* (2006) emphasized removal of abiotic stress (salt) alleviation by employing plant growth-promoting bacteria (PGPB). Under stress, IAA is responsible for an increase in root length with surface area is responsible for enhanced uptake of

available nutrients (Egamberdieva and Kucharova, 2009). Excessive production of ethylene reduces plant growth which is synthesized by an enzyme ACC oxidase in plants through precursor S-adenosylmethionine under transcriptional and post-transcriptional regulation (Hardoim *et al.*, 2008). Under abiotic stress ethylene level enhanced which is unfavourable for plant growth and may be alleviated by using beneficial PGPB. Glick *et al.* (2007) elaborately emphasized role of ACC deaminase producing microbial strain that alleviate ethylene stress whereby plant survive under salt stress.

By keeping above views in mind, the objective of this research was to characterize *in vitro* plant growth-promotion characteristics of bacterial strain and effect on seed emergence of soybean under salt stress.

MATERIALS AND METHODS

Bacterial strain and inoculum preparation: A PGPB strain *Pseudomonas* sp. strain VS1 (NCBI Accession no. JF699698, Table 1) was used in this study and maintained in yeast extract mannitol broth (YEMB, HiMedia, India) amended with 50% glycerol at -80°C. PGPB inoculum was prepared by harvesting bacterial cells from 24 h cultures on YEMB plates at 26°C. The inoculum was suspended in sterile physiological saline (0.9%) water to yield 10⁸ colony forming units (cfu) per mL.

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Table 1: Plant growth-promotion (PGP) characteristics of strain with NCBI GeneBank accession no

Strain	PGP characteristics						NCBI GeneBank Accession no.
	IAA	P sol	Sid	Pro	NF	ACC-D	
VS-1 (<i>Pseudomonas</i> sp.) Recovered from root-nodule of <i>Vigna aconitifolia</i>	+	+	+	+	+	+	JF699698

All tests were performed in three replicates, where, IAA indole 3-acetic acid production, Pi: Phosphate solubilization, Sid: Siderophore production, Pro: Protease, NF: Nitrogen fixation, ACC-D: 1-aminocyclopropane-1-carboxylate deaminase

Indole 3-acetic acid (IAA) production under salt: Indole 3-acetic acid (IAA) production was detected according by method described by Gordon and Weber (1951). Quantitative analysis of IAA was performed with concentration 500 ppm filter-sterilized L-TRP (Millipore membranes; pore size, 0.22 µm, Millipore Corporation, Bedford, MA, USA) in presence of 200 mM NaCl. Bacterial culture was grown for 24 h in YEM broth medium. Fully grown culture was centrifuged at 5000 rpm for 10 min. One mL of supernatant was mixed with 4 mL of Salkowski's reagent (150 mL of 18 M H₂SO₄, 250 mL distilled water, 7.5 mL of 0.5M FeCl₃.6H₂O) and absorbance at 535 nm was measured after 20 min (Patten and Glick, 2002). Development of pink colour was predictive IAA production. Similarly, colour was also developed in standard solutions of IAA used to prepare a standard curve. Five independent replicates of each isolate were analyzed. Concentration of IAA produced by cultures was measured with the help of standard graph of IAA obtained in the range of 10-100 ppm.

ACC deaminase activity: ACC deaminase activity was determined by the method of Glick *et al.* (1999) with little modification. The isolate was grown in liquid DF salt medium (one liter medium contains 4 g KH₂PO₄, 6 g Na₂HPO₄, 0.2 g MgSO₄.7H₂O, 2 g glucose, 2 g gluconic acid, 2 g citric acid, 100 mg FeSO₄.7H₂O, 10 µg H₃BO₃, 11.19 µg MnSO₄.H₂O, 124.6 µg ZnSO₄.7H₂O, 78.22 µg CuSO₄.5H₂O, 10 µg MoO₃, pH 7.2) with 5 mM ACC (1-aminocyclopropane-1-carboxylic acid) as nitrogen source and 200 mM NaCl at room temperature for 72 at 120 rpm. ACC solution was filter sterilized before being added into sterilized DF salt medium. Utilization of ACC by PGPB was determined if ACC containing DF salt medium turned turbid which is considered an indication of the present of ACC deaminase.

Plant material and growth conditions: Seeds of soybean variety JS9560 were used in this study. Surface sterilized soybean seeds (treated with 0.1% HgCl₂ and 70% ethanol for three minutes and washed repeatedly with milli Q water (Millipore, Germany) were placed in the small polypropylene cups. In each cup, three soybean seeds

were placed in autoclaved soil (two times on two successive days in muslin cloth) at a distance of 1.5 cm from each other and from the cup edges. The cups were placed in a Plant growth chamber (Weiber, ACMAS, India) at 26°C and 16 h/8 h light/dark photoperiod with 80% humidity.

Salt stress treatment: A soil was used for bacterial treatment which was incubated for two days with 10⁸ colony forming units (cfu) per mL. A salt concentration (200 mM) was applied at day-0. A salt solution of 50 mL was applied to each pot three days in a row without watering. The experiment was designed as a randomized complete block with PGPB strain and a water control. Five replications per treatment were used and the experiment was conducted three times. The percentage of soybean plants emerged was determined at 7 days after seeding (DAS). The germination (%), number of lateral roots, shoot and root dry weight, shoot and root lengths were measured after 7 DAS.

PGPB population affected by salt stress: An initial bacterial population of 10² cfu was added into 50 mL YEMB containing sterilized water, 200 mM NaCl solution for 24 h incubation at room temperature at 120 rpm. The bacterial population was calculated using the most probable number procedure. A standard ten-fold serial dilution was conducted and the numbers of positive growth responses were recorded after incubation for 4 days. Three replications per treatment were used.

Statistical analysis: All data were subjected to analysis of variance (ANOVA) using Microsoft software by using Excel sheet. Treatment means were separated by Fisher's protected Least Significant Difference (LSD) test at p = 0.05.

RESULTS

Plant growth-promotion characteristics of strain: In the present study a plant growth-promoting bacterium, *Pseudomonas* sp. strain VS1 was employed to alleviate salt stress in soybean plant (Table 1).

Bacterial effects on emergence of soybean under salt stress: Soybean seeds without PGPB treated soil were subjected to salt stress (200 mM NaCl) at 0 day and the percentage of emerged plants were recorded at 7 DAS. Seeds with PGPB treated soil were subjected to salt stress (200 mM) at 0 DAS, strain VS1 (*Pseudomonas* sp.), significantly enhanced emergence at 7 DAS than control (Fig. 1, Table 2). Strain untreated soybean plants had a 33% seed germination when 200 mM NaCl was applied

Table 2: PGPB-treated effect on different parameters of soybean seed emergence under salt stress

Treatment	Germination (%)	Root length (cm)	Lateral roots (no.)	Shoot length (cm)	Fresh weight (gm)		Dry weight (gm)	
					Root	Shoot	Root	Shoot
Control	33.0 ^a	1.00 ^a	2.00 ^a	2.5 ^a	0.90 ^a	1.20 ^a	0.045 ^a	0.375 ^a
VS1	100.0 ^b	10.00 ^b	21.00 ^b	10.0 ^b	3.80 ^b	6.00 ^b	0.850 ^b	1.200 ^b
LSD (p = 0.05)	18.9	0.21	0.56	1.2	0.33	0.24	0.320	0.690

Values are means of 5 replications, Superscripted letters indicate values within the same column that are either significantly different (when the letters are different) or not (when the letters are the same) using Fisher's LSD test at p = 0.05



Fig. 1: Effect of salt (200 mM) stress on soybean plant, a: Control (untreated), b: Strain treated plant

at 0 DAS and the root length was significantly decreased compared to the strain treated plants (LSD_{0.05} = 0.21). Strain significantly enhanced shoot length when 200 mM NaCl was applied at 0 DAS. Moreover, when 200 mM NaCl was applied at 0 DAS, strain treated soybean plants had significantly enhanced root and shoot weight. When 200 mM NaCl was applied at 0 DAS, soybean plants treated with strain VS1 had significantly greater root and shoot weights compared to the untreated plants.

Most importantly, the application of 200 mM NaCl at 0 DAS resulted in only a 9% of lateral root in untreated plants as compared to strain treated plants (Table 2).

DISCUSSION

In the present study, we selected one PGPB strain that screened on the basis of *in vitro* assays for plant growth-promotion properties such as IAA production and ACC deaminase. Our results indicate that PGPB was able to ameliorate salt stress. Patten and Glick (2002) reported

that bacterial IAA enhanced root and lateral root in host plant under stress which was further supported by Verma *et al.* (2001). Mayak *et al.* (1999) designed bioassay experiments for canola and mung bean seeds cuttings that showed significant increase of lateral and adventitious roots when inoculated with *P. putida* strain GR12-2 in comparison to uninoculated plants which was further confirmed by Patten and Glick (2002). This is the first report with production of IAA that performed with concentration 500 ppm of filter-sterilized L-TRP in presence of 200 mM NaCl. Dimkpa *et al.* (2009) described adaptation mechanisms of plants that exposed to environmental stress, viz., water, nutrient deficiency and heavy metal toxicity which reflect changes in root morphology by producing phytohormone. ACC deaminase activity in agar plate was positive for the isolate. High salt stress (200 mM) significantly reduced the emergence of soybean plants when salt was applied at the time of seeding (0 DAS). However, the percentage of emergence was significantly increased upon treatment with the *Pseudomonas* sp. strain VS1. Our results showed that provided a better growth promotion compared to control when soybean plants were under salt stress.

Our results indicated the role of bacteria in plant growth promotion characteristics. These beneficial microorganisms colonize the rhizosphere of plants and promote growth of the plants through various direct and indirect mechanisms. The plant-associated habitat is a dynamic environment in which many factors may affect the structure and species composition of the bacterial communities that colonize plant tissues. An understanding of the structure and species composition of plant-associated bacterial populations is fundamental to understanding how plant-associated biological processes are influenced by environmental factors and, consequently has important biotechnological implications.

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