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Ameliorative effect of plant growth promoting bacterial endophyte *Pantoea agglomerans* on salt stress at early stage of growth in rice

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ABSTRACT

Aims: Endophytes are known to impart tolerance to crop plants; hence the study was initiated to evaluate some plant growth promoting endophytic bacteria isolated from rice and maize for salt tolerance in rice.
Methodology and results: A total of 31 endophytic bacteria were screened for growth promoting characteristics on the basis of phosphate solubilization, nitrogen fixation, Indole acetic acid biosynthesis, production of ammonium, protease and cellulase. Only 2 *Bacillus* species and *Pantoea agglomerans* were positive for all growth characteristics and subsequently inoculated with rice seeds under controlled as well as in saline condition (NaCl) in order to evaluate the plant growth promoting (PGP) ability on the basis of germination rate and seedling vigor. The seedling grown under control condition, significantly alter the growth parameters such as germination rate, root and shoot length, fresh and dry weight along with seedling vigor index. Under saline condition, the genus *Bacillus* and *Pantoea* invariably counteracted the effect of stress; however, *P. agglomerans* showed significant differences for shoot length, fresh and dry weight. Analysis of correlation coefficient exhibited positive and strong association of seedling vigor index with dry weight and rate of germination, thereby revealing the interdependency of the traits under stress.
Conclusion, significance and impact of study: The study confirmed a mutualistic interaction of *P. agglomerans* with the host plant rice and leads to enhanced growth of the host under salt stress condition.

Keywords: Bacteria, *Bacillus* sp., endophytes, plant growth promotion, *Pantoea agglomerans*, salt stress

INTRODUCTION

Salt stress is one of the major factor significantly affects plant productivity and it emerges from increased concentration of soluble salts in the soil as well as in irrigation water. High salinization in soil most probably interferes with soil – water balance and severely inhibits plant growth due to improper nutrient absorption. Although the majority of crop species is sensitive to stress, only a small group of plants is capable of tolerating unfavorable conditions and evolved an array of adaptive mechanism (Silva Maganhotto de Souza and Fay, 2012). Studies have examined those intricate responses attributed to series of morphological, physiological and metabolic changes and demonstrated that intensity of salinity impairs vegetative as well as reproductive growth in number of ways, such as: i) reduce seed germination through osmotic effect, ii) decrease plant growth due to reduce cell division and elongation, iii) minimize stomatal conductance and photosynthetic rate, iv) premature senescence (Boubaker, 1996; Sultana *et al*., 1999; Wang *et al*., 2001; Munns, 2002; Moud and Maghsoudi, 2008; Tuteja, 2009). Use of endophytes has suggested as an alternate and effective approach to enhance salt tolerance in plants.

Endophytes are microbes living in symbiotic association with internal tissues of plant species, often originate from the soil; initially colonize the root and gradually spread to intercellular spaces in other parts of the plant (Chi *et al*., 2005; Long *et al*., 2008; Ulrich *et al*., 2008) whereby establishes a relation with host species. It has been recognized that the complementary association of plant-microbes are capable of promoting growth by managing soil and plant health (Sturz *et al*., 2000; Welbaum *et al*., 2004; Compant *et al*., 2005) either directly or indirectly. Multitude of mechanisms have been reported for plant growth promoting (PGP) activities by endophytes in terms of nitrogen fixation, phosphates solubilization from organic or inorganic phosphates; production of phytohormones such as auxins, cytokinins and gibberellins; lowering of ethylene level; sequestration of iron; synthesis of cell wall lysing enzymes etc. (Lucy *et al*., 2004; Sessitsch *et al*., 2004; Selosse *et al*., 2004; Welbaum *et al*., 2004; Compant *et al*., 2005; Shin *et al*., 2007). Besides promoting growth, endophytic colonization has also known to enhance tolerance to biotic and abiotic stresses in a broad range of hosts (Schulz and Boyle,

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2005; Cohen *et al*., 2008; Lugtenberg and Kamilova, 2009). For example, bacterial genus *Rhizobium*, *Bacillus*, *Pseudomonas*, *Burkholderia*, *Pantoea*, *Paenibacillus*, *Achromobacter*, *Azospirillum*, *Microbacterium*, *Methylobacterium*, *Variovorax*, *Enterobacter* etc., promoted the plant growth and confer tolerance to different abiotic stresses (Baltruschat *et al*., 2008; Egamberdieva *et al*., 2008; Egamberdieva and Kucharova, 2009; Mei and Flinn, 2010; Grover *et al*., 2010; Chakraborty *et al*., 2011; Turner *et al*., 2013) through osmotic adjustment, stomatal regulation, modification of root morphology, enhance uptake of minerals, reducing toxic effects of Na^+ and Cl^-. These findings have opened a new insight on enhancing sustainable and renewable source of crop production in marginal lands. Recently, increased interest has been shown in exploiting the synergistic effect of plant-endophyte partnership in counteracting environmental stresses and considerable progress has been achieved through breeding programs (Coleman-Derr and Tringe, 2014). Rice is highly sensitive to salinity and often develops visual symptoms due to accumulation of Na^+ ions. Even a low concentration of salt (20-50 mM NaCl) in soil can cause oxidative damages as well as ion toxicity (Greenway and Munns, 1980). Further, the effect of stress is more vulnerable during emergence and early development stages, therefore it is essential to screen the plant species for tolerance at early stages of growth. The present study was conducted in the laboratory with an aim to examine the plant growth promoting ability of isolated endophytic bacteria and their potential role in enhancing seedling vigor in rice under sodium chloride (NaCl) induced salinity stress.

MATERIALS AND METHODS

A total of 31 endophytic bacterial strains which were previously isolated from different rice (*Oryza sativa* L.) and maize (*Zea mays*) fields in Malaysia formed the study material. The samples identified using 16S rRNA gene analysis were obtained from Dr. Subhash J. Bhore, Department of Biotechnology, Faculty of Applied Sciences, AIMST University, Malaysia. The identity of the isolates was published by Loh *et al*. (2013) and the details of the material used in the study are furnished in Table 1. The isolates were screen for growth promoting activities like phosphate solubilization, nitrogen fixation, Indole acetic acid biosynthesis, production of ammonium**,** protease and cellulase. Each test was replicated thrice.

Study of growth promoting activities of bacterial endophytes

Phosphate solubilization by endophytes

Phosphate solubilization was conducted qualitatively by plating the endophytic bacterial strains bacterium in Pikovskaya medium to produce a precipitate of $CaHPO_4$ (Katzelson and Bose, 1959). All the endophytic bacterial strains were inoculated in plate containing Pikovskaya's agar medium. Each bacterial culture was spot inoculated in the center of a plate and incubated at 28 ± 2 °C for 7 days. Phosphate solubilization was assessed by the appearance of transparent halos around each colony.

Nitrogen fixing activity

Nitrogen fixing activity was conducted qualitatively by plating the endophytic bacterial isolates in Glucose Nitrogen Free Mineral (GNFM) agar medium with Bromothymole Blue (BTB) incubated at 28 ± 2 °C for 7 days (Ahmad *et al*., 2013). Atmospheric nitrogen fixation activity was assessed by color change.

Production of indole acetic acid

IAA production was detected according to the method described by Brick *et al*. (1991). The isolates were inoculated in mineral salt medium supplemented with L-tryptophan and incubated at 28 ± 2 °C for 5 days. A portion of fully grown cultures (5 mL) were removed from each tube and centrifuged at 1000 rpm for 10 min. The supernatant (2 mL) was mixed with one drop of orthophosphoric acid and 2 mL of the Salkowski reagent. The mixture was incubated at room temperature for 25 min and development of pink color indicates the production of IAA.

Production of ammonium

Ammonium production was determined as suggested by Cappuccino and Sherman (1992). Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 mL peptone water in each tube and incubated at 28 °C for 48 h. After incubation, Nessler's reagent (0.5 mL) was added to each tube. The development of colour from yellow to brown indicates a positive test for ammonia production.

Production of protease

Protease activity was evaluated on skimmed milk agar medium (Carrim *et al*., 2006). After autoclaving, 100 mL of skimmed milk were prepared, separately autoclaved and added to LB agar medium. The bacterial isolates were inoculated in skimmed milk agar medium and incubated at 28 °C for 48 h. After the growth period, 2.0 mL of HCl 0.1 mol/L was added to the plates and the presence of clear halos around the colonies was observed.

Cellulase activity of endophytes

Cellulase production was determined by the method of Andro *et al*. (1984). Pure cultures of bacterial isolates were individually transferred in CMC agar plates. After incubation for 48 h, CMC agar plates were flooded with 1% congo red and allowed to stand for 15 min at room temperature. One molar NaCl was used for counterstaining the plates. Clear zones appeared around growing bacterial colonies indicating cellulose hydrolysis.

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Table 1: List of endophytic bacteria isolated from rice and maize plants from various states of peninsular Malaysia.

Isolate code	Endophytic bacterial isolates	Cereal crops	Organ	State	Accession No	Identity %
1.23.1	*Bacillus anthracis*	*Zea mays*	Leaf	Kedah	HQ694462	100
1.OS(CY).1	*Bacillus thuringiensis*	*Oryza sativa*	Leaf	Kedah	HQ694036	100
1.OS(CY).2	*Bacillus anthracis*	*Oryza sativa*	Leaf	Kedah	HQ694037	100
1.OS(CY).4	*Bacillus koreenis*	*Oryza sativa*	Leaf	Kedah	HQ694039	100
1.OS(CY).5	*Bacillus cereus*	*Oryza sativa*	Leaf	Kedah	HQ694040	99
1.OS(CY).6	*Bacillus koreenis*	*Oryza sativa*	Leaf	Kedah	HQ694041	98
1.OS(CY).7	*Bacillus koreenis*	*Oryza sativa*	Leaf	Kedah	HQ694042	100
1.OS(CY).8	*Bacillus koreenis*	*Oryza sativa*	Leaf	Kedah	HQ694043	100
1.OS(CY).9	*Bacillus megaterium*	*Oryza sativa*	Leaf	Kedah	HQ694044	100
1.OS(CY).14	*Bacillus cereus*	*Oryza sativa*	Leaf	Kedah	HQ694049	99
1.OS(CY).15	*Bacillus subtilis*	*Oryza sativa*	Leaf	Kedah	HQ694050	100
1.OS(CY).17	*Bacillus subtilis*	*Oryza sativa*	Leaf	Kedah	HQ694052	100
1.OS(CY).18	*Bacillus cereus*	*Oryza sativa*	Leaf	Kedah	HQ694053	100
1.OS(CY).19	*Bacillus thuringiensis*	*Oryza sativa*	Leaf	Kedah	HQ694054	100
2.OS.2	*Pantoea agglomerans*	*Oryza sativa*	Leaf	Perlis	HQ694067	100
2.OS.3	*Pantoea agglomerans*	*Oryza sativa*	Leaf	Perlis	HQ694068	100
3.24.1	*Pantoea dispersa (Enterobacter)*	*Zea mays*	Leaf	Penang	HQ694033	100
3.24.2	*Bacillus megaterium*	*Zea mays*	Leaf	Penang	HQ694028	98
4.P2.1	*Bacillus cereus*	*Zea mays*	Leaf	Perak	HQ694024	100
4.P2.4	*Bacillus cereus*	*Zea mays*	Leaf	Perak	HQ694027	100
4.P2.5	*Pantoea agglomerans*	*Zea mays*	Leaf	Perak	HQ694032	99
5.K5.1	*Bacillus megaterium*	*Oryza sativa*	Leaf	Selangor	HQ694058	100
5.K5.2	*Bacillus pumilus*	*Oryza sativa*	Leaf	Selangor	HQ694059	100
6.N2.2	*Bacillus cereus*	*Zea mays*	Leaf	Negeri Sembilan	HQ694034	100
7.M2.1	*Bacillus anthracis*	*Oryza sativa*	Leaf	Melaka	HQ694060	100
7.M2.2	*Bacillus megaterium*	*Oryza sativa*	Leaf	Melaka	HQ694069	100
10.T2.1	*Bacillus subtilis*	*Zea mays*	Leaf	Terengganu	HQ694030	100
10.T10.1	*Lysinibacillussphaericus (Bacillus)*	*Oryza sativa*	Leaf	Terengganu	HQ694063	100
11.K2.2	*Bacillus pumilus*	*Zea mays*	Leaf	Kelantan	HQ694031	100
11.K10.1	*Bacillus subtilis*	*Oryza sativa*	Leaf	Kelantan	HQ694065	100
11.K10.2	*Bacillus pumilus*	*Oryza sativa*	Leaf	Kelantan	HQ694066	100

Source: Loh *et al*. (2013).

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Screening of endophytes for salinity tolerance

Rice seeds (MR 219) were surfaced sterilized in 2% (v/v) sodium hypochlorite for 15 min and washed thoroughly with sterile distilled water. Bacterial suspensions in sterile distilled water (10^8 CFU/mL) were used for seed inoculation. Experiment consists of the following treatment with rice seeds: a) bacterial inoculation (C + EBI), b) without bacterial inoculation (C), c) bacterial inoculation with salt stress (NaCl + EBI) and d) without inoculation with salt stress (NaCl).

The seeds were incubated at room temperature overnight and allowed to germinate on a moist filter paper (Whatman No.1) in Petri dishes. A solution of NaCl (150 mM) was prepared and applied to the seeds to assess the effect of salt stress in rice. The experiments were conducted in completely randomized design with three replications; a Petri dish containing 25 seeds serves as a replicate. On the 10^{th} day after germination, five seedlings from each replicates were selected to evaluate the effect of bacterial endophytes on seedling vigor and plant growth. Fresh weight (FW) of the seedlings was obtained and then the root and shoot portions were separated to measure their length. Dry weight was determined by drying the seedling in an incubator at 48 °C for 2 days. After achieving constant weight, the final weight of dried leaf samples was taken using a laboratory scale. Seedling vigor index was determined by following Abdul-Baki and Anderson (1973).

Statistical analysis

The data collected were calculated for appropriate standard error of the mean and the result is presented in graph using Microsoft Excel. Analysis of variance was carried out using statistical software (SPSS V.20.0). LSD test was used to compare the mean of root length, shoot length, fresh weight, dry weight and seedling vigor index. Correlation coefficient analysis (r) was used to compare the relationship between growth parameters and seedling vigor.

RESULTS AND DISCUSSION

Selection of potential plant growth promoting endophytic isolates

A total of 31 bacterial endophytes isolated from rice and maize plants were used to assess their growth promoting activities in chemically defined medium. The majority of them are *Bacillus* genus and the qualitative estimation of PGP activities of those isolates (Tables 2 and 3) revealed that 26 bacterial strains have the ability to solubilize phosphate as it formed a clear zone around the colonies, 20 showed nitrogenous activity reflecting their capability to fix free nitrogen, 16 were able to produce auxin, 23 produced ammonia and also capable of hydrolyzing cellulose, 25 synthesis protease. Among the bacterial isolates screened, only four strains such as *Bacillus anthracis* (Isolate code: 1.23.1 from maize and 7.M2.1 from rice), *B. megaterium* (Isolate code: 3.24.2 from maize) and *Pantoea agglomerans* (Isolate code: 4.P2.5 from maize) displayed positive for all the six PGP activities.

The effect of nitrogen fixation and phosphate solubilization seems to be the most important mechanism of the isolates because N and P are vital for the functionality of plant growth. Further, production of IAA by the strains is capable of promoting cell division, cell elongation and cell differentiation; in addition to vascular development and lateral root formation. Positive for protease and cellulase production indicated the effectiveness of the isolates in invasion and colonization besides imparting tolerance under adverse conditions. Hence those four isolates were co-cultivated with rice seeds and further evaluated for growth promoting ability on the basis of germination rate and seedling vigor. It is evident from the result (Figures 1 and 2) that endophytic partnership (C+ EBI) invariably accelerated the seedling growth, however the strain; *P. agglomerans* (4.P2.5) had shown an increased rate of shoot (72.4%) and root growth (31.7%) as well as fresh weight (57.4%) when compared to un-inoculated control (C). There have been a number of evidences on the contribution of PGP activity of those two bacterial strains (Rodríguez and Fraga, 1999; Richardson, 2001; Kloepper *et al*., 2004; Esitken *et al*., 2006; Gotz *et al*., 2006; Dursun *et al*., 2010). The finding is in agreement with the earlier reports and thus demonstrated the capability of the genus *Bacillus* and *Pantoea* in facilitating plant growth by contributing N, phosphorus and IAA.

Identification of bacterial strain for salt stress tolerance

The effect of salt stress (NaCl) in rice is presented in Figures 1 and 2, indicate that salinity had a negative influence on the rate of germination (19.2%), emergence of root (96.8%) and shoot (91.4%) growth, fresh (54.1%) and dry (29.1%) weight as against control (C). Examination on the effect of salt stress had shown a drastic reduction for growth parameters and seedling vigor in rice seedling. Decrease in growth activity might be due to increased ionic toxicity which inhibits water holding capacity and impair physiology processes of the seedling (Bouhmouch *et al*., 2005). Extensive studies have been made on the effect of salinity stress in growth and development of diverse crop species. Use of microorganisms proposed by several authors (Barassi *et al*., 2009; Egamberdieva, 2009; Kang *et al*., 2009) has drawn attention to elucidate the ameliorative effect of salt stress. The rice seeds was inoculated with four isolates (*B. anthracis* [1.23.1 and 7.M2.1], *B. megaterium* and *P. agglomerans*) and analyzed for stress tolerance after the application of NaCl. The result seems to be encouraging as the endophytic isolates significantly improved the seedling growth (NaCl + EBI) and it was evident from the Figure 1 and 2. Inoculation of the strains improved the rate of germination (ranged from 17.6 % to 20.6 %), root

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Table 2: Screening of endophytic bacteria isolated from rice and maize plants for plant growth promoting activities like Phosphate solubilization, Nitrogen fixation and Indole acetic acid (IAA) production.

S. No	Isolate code	Endophytic bacterial isolates	Phosphate solubilization	Nitrogen fixation	Indole acetic acid (IAA)
1.	1.23.1	*Bacillus anthracis*	+	+	+
2.	1.OS(CY).1	*Bacillus thuringiensis*	+	+	-
3.	1.OS(CY).2	*Bacillus anthracis*	+	-	-
4.	1.OS(CY).4	*Bacillus koreenis*	-	-	+
5.	1.OS(CY).5	*Bacillus cereus*	+	+	-
6.	1.OS(CY).6	*Bacillus koreenis*	+	+	+
7.	1.OS(CY).7	*Bacillus koreenis*	+	+	+
8.	1.OS(CY).8	*Bacillus koreenis*	-	-	-
9.	1.OS(CY).9	*Bacillus megaterium*	-	+	+
10.	1.OS(CY).14	*Bacillus cereus*	+	-	-
11.	1.OS(CY).15	*Bacillus subtilis*	+	-	-
12.	1.OS(CY).17	*Bacillus subtilis*	-	+	+
13.	1.OS(CY).18	*Bacillus cereus*	+	+	-
14.	1.OS(CY).19	*Bacillus thuringiensis*	+	-	-
15.	2.OS.2	*Pantoea agglomerans*	+	+	+
16.	2.OS.3	*Pantoea agglomerans*	+	+	+
17.	3.24.1	*Pantoea dispersa (Enterobacter)*	+	+	+
18.	3.24.2	*Bacillus megaterium*	+	+	+
19.	4.P2.1	*Bacillus cereus*	-	+	-
20.	4.P2.4	*Bacillus cereus*	+	-	+
21.	4.P2.5	*Pantoea agglomerans*	+	+	+
22.	5.K5.1	*Bacillus megaterium*	+	+	+
23.	5.K5.2	*Bacillus pumilus*	+	+	-
24.	6.N2.2	*Bacillus cereus*	+	+	-
25.	7.M2.1	*Bacillus anthracis*	+	+	+
26.	7.M2.2	*Bacillus megaterium*	+	-	+
27.	10.T2.1	*Bacillus subtilis*	+	+	-
28.	10.T10.1	*Lysinibacillussphaericus (Bacillus)*	+	+	-
29.	11.K2.2	*Bacillus pumilus*	+	-	+
30.	11.K10.1	*Bacillus subtilis*	+	-	-
31.	11.K10.2	*Bacillus pumilus*	+	-	-

+, Presence; -, absence of activity.

and shoot emergence (1.59% - 1.7% and 13.8% - 20.69%), fresh (9.84% - 14.75%), dry weight (16.67% - 20.83%) and seedling vigor (33.84% - 37.9%) respectively.

Leven's test confirmed equal variances among the group and one way ANOVA showed (Figures 1 and 2) significant differences ($p < 0.05$) between treatments and for growth parameters. Though endophytic inoculation with NaCl recorded significant differences for growth parameters; comparison of mean using LSD had shown significant difference ($p < 0.05$) for the strain 4.P2.5 (*P. agglomerans*) for traits like shoot length, fresh and dry weight as against salt stress (Figures 1 and 2). Growth promotion activity observed under stress condition is primarily due to synthesis of hydrolytic enzymes by the endophytes which accumulates osmolytes and improved the water status. Increased water status had accelerated hydrolysis of food reserve and enhances seedling emergence as well as establishment (Dubey, 2003). Further, co-cultivation of endophytes had counteracted the inhibitory effect of salinity on nitrogen fixation and increased the supply of N (Egamberdieva, 2009). It also assists hydrolysis of acid phosphatases to release assimilable from of P to maintain ionic balance. Furthermore, endophytic partnership activates 1-aminocyclopropane carboxylate (ACC) deaminase to regulate ethylene biosynthesis, ammonia thus released (Glick, 2004; Lugtenberg and Kamilova, 2009) increased the root and shoot growth. According to Figueiredo *et al*. (2008), stress responses in plants are mediated by phytohormones which regulate stomatal openings and maintain the rate of metabolism. Elevated performance of the inoculation is obviously reflected in increase seedling vigor index. This is mainly due to increased mobility of nitrogen in plant system increased the dry matter accumulation compared to salt induced seedlings.

Analysis of correlation coefficient revealed a significant and positive association among the growth parameters studied (Table 4). It is evident from the result that the seedling vigor under salt stress condition is greatly dependent on dry weight ($r = 0.97$) and the rate of germination ($r = 0.62$) and the result is in agreement with the earlier findings (Rajendran *et al*., 2007; Long *et al*., 2008). Among the strains studied, *Pantoea agglomerans*

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alone had brought marked changes in plant growth under salt stress condition. Studies have confirmed the growth promoting ability of this enterobacter (Verma *et al*., 2004; Esitken *et al*., 2006; Gotz *et al*., 2006; Dursun *et al*., 2010) in various crop species under normal condition.

Table 3: Screening of endophytic bacteria isolated from rice and maize plants for the production of ammonia, protease and cellulose.

S. No.	Isolate code	Endophytic bacterial isolates	Production of		
			Ammonia	Proteasa	Cellulase
1.	1.23.1	*Bacillus anthracis*	+	+	+
2.	1.OS(CY).1	*Bacillus thuringiensis*	+	+	-
3.	1.OS(CY).2	*Bacillus anthracis*	-	+	-
4.	1.OS(CY).4	*Bacillus koreenis*	+	+	+
5.	1.OS(CY).5	*Bacillus cereus*	+	+	+
6.	1.OS(CY).6	*Bacillus koreenis*	-	-	+
7.	1.OS(CY).7	*Bacillus koreenis*	+	+	-
8.	1.OS(CY).8	*Bacillus koreenis*	+	+	+
9.	1.OS(CY).9	*Bacillus megaterium*	-	+	+
10.	1.OS(CY).14	*Bacillus cereus*	+	+	+
11.	1.OS(CY).15	*Bacillus subtilis*	-	+	+
12.	1.OS(CY).17	*Bacillus subtilis*	+	+	-
13.	1.OS(CY).18	*Bacillus cereus*	+	-	+
14.	1.OS(CY).19	*Bacillus thuringiensis*	+	+	-
15.	2.OS.2	*Pantoea agglomerans*	+	-	+
16.	2.OS.3	*Pantoea agglomerans*	+	-	+
17.	3.24.1	*Pantoea dispersa (Enterobacter)*	+	-	+
18.	3.24.2	*Bacillus megaterium*	+	+	+
19.	4.P2.1	*Bacillus cereus*	+	+	+
20.	4.P2.4	*Bacillus cereus*	+	+	+
21.	4.P2.5	*Pantoea agglomerans*	+	+	+
22.	5.K5.1	*Bacillus megaterium*	+	-	+
23.	5.K5.2	*Bacillus pumilus*	+	+	+
24.	6.N2.2	*Bacillus cereus*	+	+	+
25.	7.M2.1	*Bacillus anthracis*	+	+	+
26.	7.M2.2	*Bacillus megaterium*	-	+	+
27.	10.T2.1	*Bacillus subtilis*	-	+	-
28.	10.T10.1	*Lysinibacillussphaericus (Bacillus)*	+	+	-
29.	11.K2.2	*Bacillus pumilus*	-	+	+
30.	11.K10.1	*Bacillus subtilis*	+	+	-
31.	11.K10.2	*Bacillus pumilus*	-	+	+

+, Presence; -, absence of activity.

Table 4: Correlation coefficient (r) for growth parameters assessed under NaCl induced stress condition in rice.

	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	Germination (%)	Seedling Vigor Index
Shoot length (cm)	1	0.91*	0.60*	0.25*	0.50*	0.35*
Root length (cm)		1	0.56*	0.25*	0.47*	0.34*
Fresh weight (g)			1	0.28*	0.30*	0.32*
Dry weight (g)				1	0.42*	0.97*
Germination (%)					1	0.62*
Seedling Vigor Index						1

Significance at $p < 0.05$.

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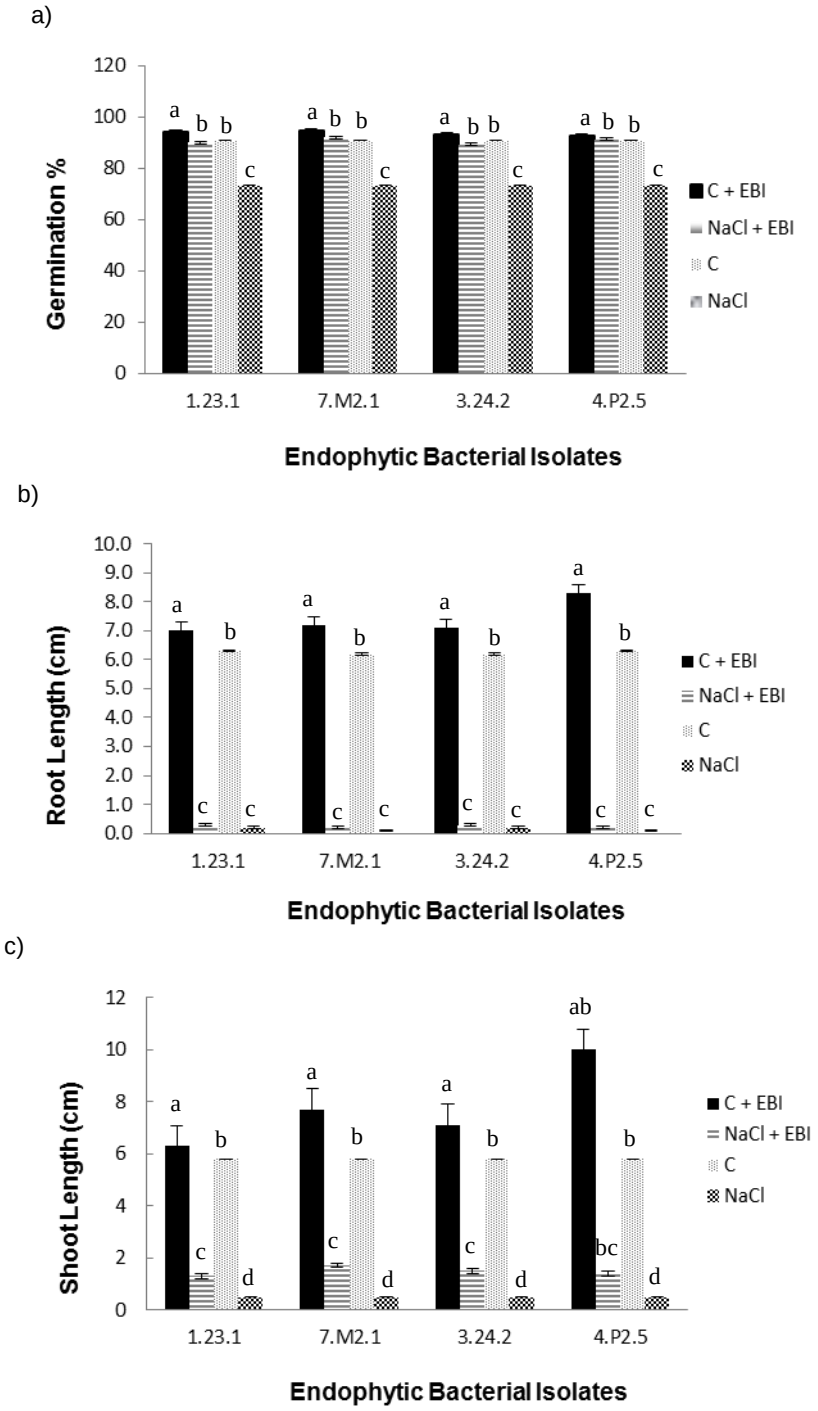

Figure 1: Effect of endophytic plant growth promoting activities of the genus *Bacillus* and *Pantoea* on a) germination (%); b) root length (cm) and c) shoot length (cm) for control and NaCl induced salt stress in rice. Experiments were performed in triplicate. Values indicated by different letters are significantly different at $p < 0.05$. C + EBI: bacterial inoculation, C: without bacterial inoculation; NaCl + EBI: bacterial inoculation with salt stress and NaCl: without inoculation with salt stress.

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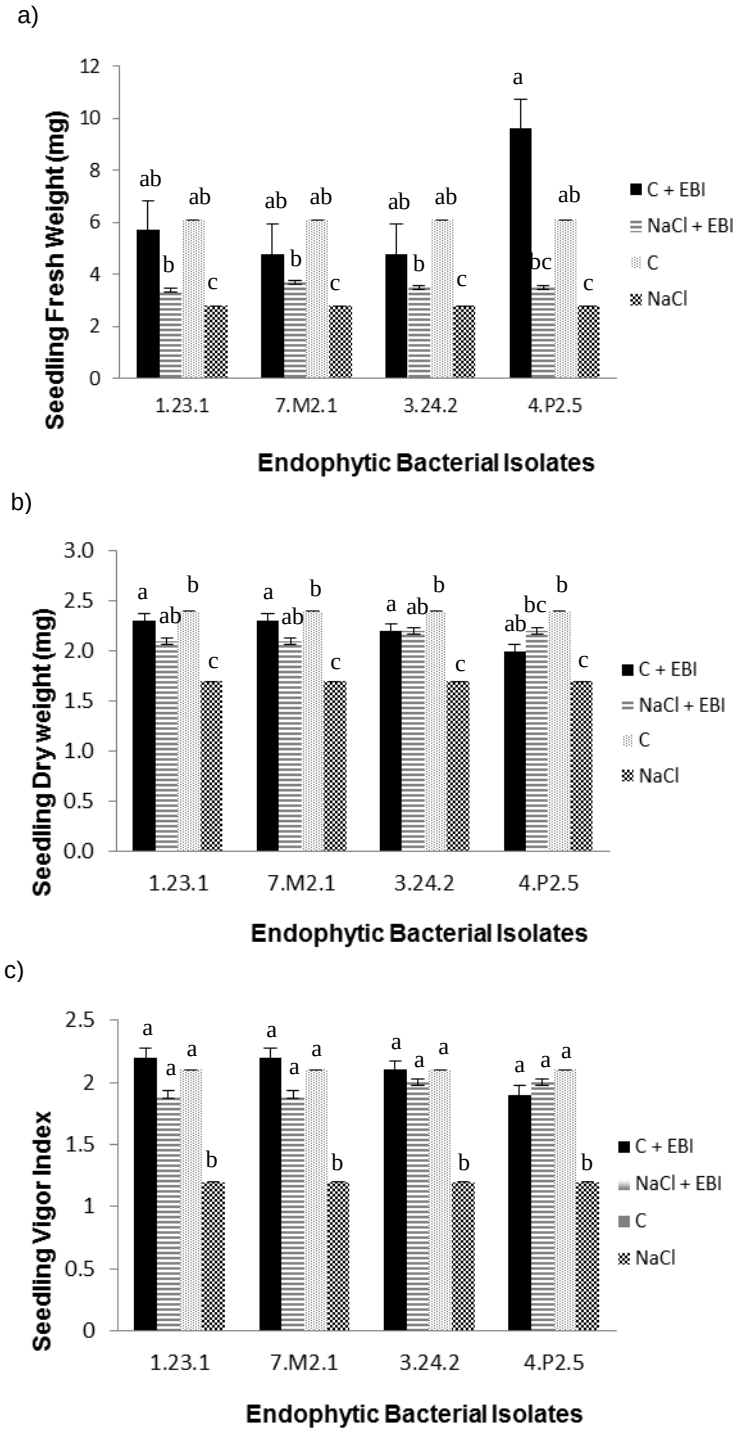

Figure 2: Effect of endophytic plant growth promoting activities of the genus *Bacillus* and *Pantoea* on a) fresh weight (mg); b)); dry weight (mg) and c) seedling vigor index for control and NaCl induced salt stress in rice. Experiments were performed in triplicate. Values indicated by different letters are significantly different at $p < 0.05$. C + EBI: bacterial inoculation, C: without bacterial inoculation; NaCl + EBI: bacterial inoculation with salt stress and NaCl: without inoculation with salt stress.

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CONCLUSION

It is concluded that the endophytic bacteria *Pantoea agglomerans* isolated from maize had shown growth promoting characteristics such as nitrogen fixation, phosphate solubilization, Indole acetic acid biosynthesis, production of ammonium, protease and cellulose. It significantly enhances the growth parameters when inoculated with rice seeds under controlled environment and also contributed significantly in ameliorating salt stress by increased shoot length, fresh weight and dry weight through increased uptake of nitrogen as well as high accumulation of dry matter compared to salt induced seedlings under laboratory condition. Further, *P. agglomerans* has a potential to sustain plant growth under stress and hence it can be used in seed treatment to mitigate the effect of salt stress.

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