Journal of Experimental Biology and Agricultural Sciences, August - 2023; Volume – 11(4) page 746 – 755

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Halotolerant Plant Growth Promoting Bacilli from Sundarban Mangrove Mitigate the Effects of Salinity Stress on Pearl Millet (*Pennisetum glaucum* L.) Growth

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Received – June 07, 2023; Revision – August 09, 2023; Accepted – August 25, 2023
Available Online – August 31, 2023

DOI: http://dx.doi.org/10.18006/2023.11(4).746.755

KEYWORDS

Pearl millet

Salt stress

PGPR

Antioxidants

Bacillus

ABSTRACT

Pearl millet (*Pennisetum glaucum* L.) is one of the major crops in dry and saline areas across the globe. During salinity stress, plants encounter significant changes in their physio and biochemical activities, leading to decreased growth and yield. *Bacillus* species are used as biofertilizers and biopesticides for pearl millet and other crops to promote growth and yield. The use of *Bacillus* in saline soils has been beneficial to combat the negative effect of salinity on plant growth and yield. In this context, the present study emphasizes the use of two *Bacillus* species, i.e. *Bacillus megaterium* JR-12 and *B. pumilus* GN-5, which helped in alleviating the impact of salinity stress on the growth activities in salt-stressed pearl millet. Pearl millet seeds were treated with two strains, *B. megaterium* JR-12 and *B.pumilus* GN-5, individually and in combination under 50, 100 and 150 mM of sodium chloride stress. The treated plants showed higher plant height, biomass accumulation, and photosynthetic apparatus than the non-treated plants. Additionally, the treated plants showed increased osmoprotectant levels under salinity stress compared to control plants. The antioxidant enzyme content was improved post-inoculation, indicating the efficient stress-alleviating potential of both strains of *Bacillus* species. Moreover, inoculation of these microbes significantly increased plant growth attributes in plants treated with a combination of Bp-GN-5 + Bm-JR-12 and the reduction rates of plant growth were found to be alleviated to 9.12%, 20.30% and 33%, respectively. Overall, the results of the present study suggested that these microbes could have a higher potential to improve the productivity of pearl millet under salinity stress.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

Pearl millet (*Pennisetum glaucum*) is an important crop contributing to global nutritional security. It belongs to the family Gramineae and serves as a staple source of food and fodder in millions of poor households under low rainfall conditions. With rising concerns over widespread deficiency of nutrients in most of the world population, millet crops are a viable option to replace major cereals. Conversely, the ability of pearl millet to grow in poor, infertile soils makes it the best choice to grow in soils that cannot be used for other cereal crops (Kumar et al. 2010).

Salt stress is among the prominent abiotic factors limiting global agricultural productivity (Munns and Tester 2008; Shahbaz and Ashraf 2013). High sodium and chloride content interferes with the activities of various vital enzymes and thus affects plant physiology (Munns and Tester 2008). This leads to a reduction in overall productivity and yield of salt-sensitive crops and ultimately results in consumable diets low in nutrients. Millet crops generally tolerate up to 6 dS m^{-1} of salinity levels in the soil (ECe) without substantially losing dry matter. However, higher salinity levels have been reported to cause damage to the soil, especially by organic matter decomposition, nitrification, denitrification, microbial activity, and biodiversity (Schirawski and Perlin 2017; Upadhyay et al. 2019). Removing excessive sodium from the soil by conventional physical and chemical methods is an unsustainable and time-consuming process that is unsustainable and stands ineffective with higher salt concentrations (Ayyam et al. 2019). Salt stress can significantly reduce the productivity of various crops (Toro et al. 2021). However, a salinity level above ECe of 9 dS m^{-1} is reported to reduce plant productivity significantly (Evans 2006). The effect of salinity on the morphology, anatomy and physiology of pearl millet is well documented (Hussain et al. 2008, 2010). Thus, it is important to consider strategies to enhance salinity tolerance in pearl millet to maintain or increase its production under saline infertile soils.

The term "plant-growth-promoting rhizospheric bacteria" (PGPR) refers to a class of microorganisms that colonize the roots of plants or exist in the rhizosphere as free-living organisms and promote the plant growth by direct and indirect methods (Dodd and Perez-Alfocea 2012; Orhan 2016; Bhat et al. 2020). Some processes by PGPRs, such as organic acids production, which can solubilize minerals and break organic matter, P-solubilization, K-solubilization, Zn-solubilization, siderophore production for iron chelation, indole acetic acid (IAA) production for cell elongation, HCN production as a defensive compound help in the overall growth and productivity of plants (Kumar and Gera 2014). This study hypothesized that the application of halotolerant bacteria could enhance growth under high levels of salt stress conditions and focused on exploring the PGPR properties of potential halophile bacteria isolated from the natural salt-affected soils of the Sundarbans mangrove region of West Bengal, India, and to evaluate the potential of PGPR strains to alleviate effect of salinity stress on pearl millet cultivar.

2 Materials and Methods

2.1 Source of inoculum

Potential PGPR strains (*B. megaterium* JR-12 and *B. pumilus* GN-5) were obtained from the rhizospheric soil of mangrove plants thriving in saline circumstances in Sundarbans, West Bengal, India (Pallavi et al. 2023).

2.2 Plant Growth Promoting Assays

2.2.1 Screening for phosphate solubilization

The test colonies of the isolates were spot inoculated on Pikovskaya's agar medium (Pikovskaya 1948), followed by incubation at 28 ±2°C for 48 hours. Clear solubilization zones surrounding the colonies were considered positive for P-solubilization. The formula presented in Edi-Premono et al. (1996) was used to compute the solubilization index by considering the colony diameter and the diameter of the halo zone.

Phosphate Solubilization Index (PSI) = Colony diameter + Halo zone diameter /Colony diameter

2.2.2 Quantitative phosphate solubilization

By measuring the total amount of soluble phosphate in the cell-free supernatant of Pikovskaya's broth supplemented with 0.5% TCP, the quantitative measurement of phosphate solubilization efficiency of chosen PGPRs was evaluated. The amount of accessible phosphate in the culture supernatant was assessed using the phosphomolybdate technique (Watanabe and Olsen 1965).

2.2.3 IAA production potential

The IAA production potential of the selected PGPR strains was determined by the method described by Brick et al. (1991). Two sets of 25 mL nutrient broth were inoculated with 24h old culture (with and without tryptophan) and incubated at 37±2°C for 36h at 120 rpm in an incubator shaker. The IAA production (µg/mL) was determined using the standard plot of IAA.

2.2.4 Siderophore production

The qualitative analysis of siderophore production was done by the Chrome Azurol Sulfonate (CAS) method (Pérez-Miranda et al. 2007). Schwyn and Neilands (1987) method was followed to prepare CAS agar plates, which were spot inoculated by the potential PGPR isolates and incubated for 48 h (37 ± 2°C) to observe the yellow-orange halo around the colonies.

2.2.5 Zinc Solubilization Potential

The zinc solubilization efficiency of the isolates was assessed using Zinc oxide (ZnO). The 24 h old colonies were aseptically spot inoculated on respective zinc-supplemented plates (amended with 1% ZnO). The plates were incubated at 37 ± 2°C in the dark for seven days, and clear zones surrounding the colonies of zinc solubilizing isolates were observed. The zone diameters were recorded (Sharma et al. 2012).

2.2.6 Ammonia Production

The ammonia production was assessed in peptone broth following the protocol of Cappuccino and Sherman (1992). Bacterial isolates were inoculated for culture in 10 mL of peptone broth. They were further incubated at 37 ± 2°C (for 48 h), to which 0.5 mL of Nessler's reagent was added and observed for the colour change from brown to yellow, indicating positive for ammonia production.

2.3 *In planta* testing of plant growth promotion

The plant growth-promoting potential of the PGPR isolates *B. pumilus* GN-5 and *B. megaterium* JR-12 individually and in combination was tested in pearl millet (*Pennisetum glaucum* L.) cultivar Pusa composite 443.

2.3.1 Inoculation of seeds

Seeds were surface sterilized for 3 minutes with 1.2% sodium hypochlorite, rinsed thrice with sterile water and dried at room temperature (Sahu et al. 2022). Bacterial isolates were cultured in 500 mL of nutrient broth at 37°C for 48 h and centrifuged at 10,000 rpm for 10 minutes at 4°C. The pellets were rinsed and suspended in sterilized water. The final OD was adjusted to 0.8 at 600 nm (approx. 2 x 10^8 CFU/mL). The inoculum was applied to the seeds @ 2mL per kg seed, while in control treatments, seeds were treated with sterile nutrient broth in place of the bacterial suspension.

2.3.2 Effects of NaCl and bacterial Inoculation on pearl millet growth

The effects of salt stress and PGPR treatment on the pearl millet variety (Pusa Composite 443) were assessed in pot experiments under random block design in triplicates. Three kgs of soil with an initial pH of 7.2 and EC of 0.92 were supplemented with 0, 8.79, 17.55 and 26.46 g of sodium chloride dissolved in 300 mL of water to achieve salt concentrations of 0, 2.93, 5.85 and 8.82 g/kg soil, respectively. The treated seeds were planted in salt-amended pots in the greenhouse at 25 ± 2°C with RH maintained at 50% and 12:12 hour light: dark cycle. The electrical conductivity of the soil extract (obtained by dissolving 30g of dry soil in 20 mL of deionized water and agitated for an hour) was measured by a conductivity meter. The moisture content of the soil was maintained at 25% by watering the pots twice with deionized water. The plants were thinned to 5 per pot after 10 days and harvested at 45 days to observe phenotypic parameters.

2.4 Plant growth parameters

The plants were harvested after 45 days to assess growth parameters in control and PGPR-treated plants under salt stress. The total biomass accumulation was evaluated by plant dry weight by oven-dried samples at 65±5 °C for 5 days to reach constant weight. The relative water content of the leaf was measured as per the protocol given by Sairam et al. (2002) and was calculated by the following equation used to determine the relative water content of the leaf.

$$RWC\ (\%) = [(FW - DW)/(TW - DW)] \times 100$$

Here FW - fresh weight, TW - turgid fresh weight after 24 h, DW - dry weight

The chlorophyll content (Chl a and Chl b) in leaves was determined using acetone extraction followed by spectrophotometry described by Arnon (1949). The contents of Chl a, Chl b, and total chlorophyll were determined by using the formula given in Sadasivam and Manickam (1996).

2.5 Determination of proline, total reducing sugar contents, carbohydrate content and total soluble protein content

To determine the proline content in pearl millet leaves, 0.5 g of fresh leaves were crushed in liquid nitrogen and homogenized using 10 mL of 3% sulfosalicylic acid. The amount of proline (µmol/g FW) was determined by taking absorbance at 520 nm using a standard curve (Bates et al. 1973). For total reducing sugar estimation, the DNSA method was followed (Miller1959). Absorbance was taken at 540nm. The carbohydrate content determination was carried out by the method of Yemm and Wills (1954). The Bradford (1976) method was used to estimate the total protein concentration in plant leaves. The absorbance was recorded at 595nm.

2.6 Antioxidant Enzyme Assays: Superoxide dismutase (SOD) and Catalase (CAT)

To determine the antioxidant enzyme superoxide dismutase, 0.5 g fresh leaves were homogenized in 2 mL of 50 mM SOD extraction buffer and assessed activity (Dhindsa and Thorpe 1981).

The reaction mixtures of 50 mM phosphate buffer and individual enzyme extracts were prepared for the catalase activity. The enzyme activity was determined by measuring the decrease in absorbance (ΔE, min-1·g-1·FW) 240 nm (Aebi 1984).

Table 1 Plant growth promotion traits of salt tolerant *Bacillus* species

S. No	Strain	NCBI Accession	IAA Production	Zinc Solubilization	Phosphate Solubilization		Siderophore Production	Ammonia Production
					MgP_2O_5/ 100ml (8th Day)	MgP_2O_5/ 100ml (15th Day)		
1.	*B. pumilus GN-5*	MK 559616	38.34±0.76	+	25.54±0.79	35.67±0.78	+	++
2.	*B. megaterium JR-12*	MK 559615	42.34±0.56	+	27.89±1.11	37.67±1.16	+	+

2.7 Statistical analysis

All the data were taken in replicates and presented as average means with standard error. The data were analyzed using the PRISM 7.0 programme. ANOVA was used for the significance of treatments, and the significant treatments were determined at p 0.001 probability level.

3 Results

3.1 PGP activity of salt-tolerant isolates

The bacterial strains Bp-GN-5 and Bm-JR-12 isolated from the rhizosphere of mangrove soil were investigated for PGP activities such as IAA production, zinc solubilization, P_i solubilization, siderophore production and ammonia production. Results of the study revealed that Bp-GN-5 solubilized 35.67 µg/ml phosphate and produce 38.34 µg/ml indole acetic acid, while Bm-JR-12 solubilized 37.67 µg/ml phosphate and produced 42.34 µg/ml IAA. Both the isolates were zinc solubilizers, ammonia producers and siderophore producers (Table 1).

3.2 Effect of salinity on growth traits

The present study investigated two PGPR strains, Bp-GN-5 and Bm-JR-12, for their role in alleviating salinity stress on plant growth promotion in pearl millet. An increase in salt concentration from 0 to 150 mM in soil was found to reduce the growth parameters in pearl millet in untreated control, but the treatment of the two PGPR strains under salt stress was found to significantly alleviate the negative effect of salt stress on plant growth parameters. It could also help to reduce the adverse effects of ion toxicity.

The plants treated with salt-tolerant PGPR strains performed better in terms of plant growth under both salinized and non-salinized conditions. The reduction of plant height in 50, 100, 150 mM salt and PGPR-treated plants compared to untreated respective control plants were 7.46%, 29.03% and 47.93%, respectively, whereas in plants treated with a combination of Bp-GN-5 + Bm-JR-12 the reduction of plant height was found to be 9.12%, 20.30% and 33% respectively (Figure 1). Similarly, the results obtained for other plant growth parameters such as shoot dry weight were also found to be reduced in control plants by 0.56%, 8.93% and 40.73% respectively, at all salt concentration treatments whereas in plants treated with combination of Bp-GN-5 + Bm-JR-12the reduction was found to be alleviated to 38.72% in 150 mM respectively (Figure 2). Whereas, in addition of PGPR individually or in combination the growth parameters were alleviated during NaCl stress (Figure 1 and 2). The effect of salinity was reflected in the loss of relative water content as indicated by the reduction in relative water content with an increase in salt concentration (Figure 3). The individual application of Bp-GN-5 and Bm-JR-12 resulted in a non-significant difference in the growth parameters. The level of both Chl a and b content in PGPR treated plants was increased

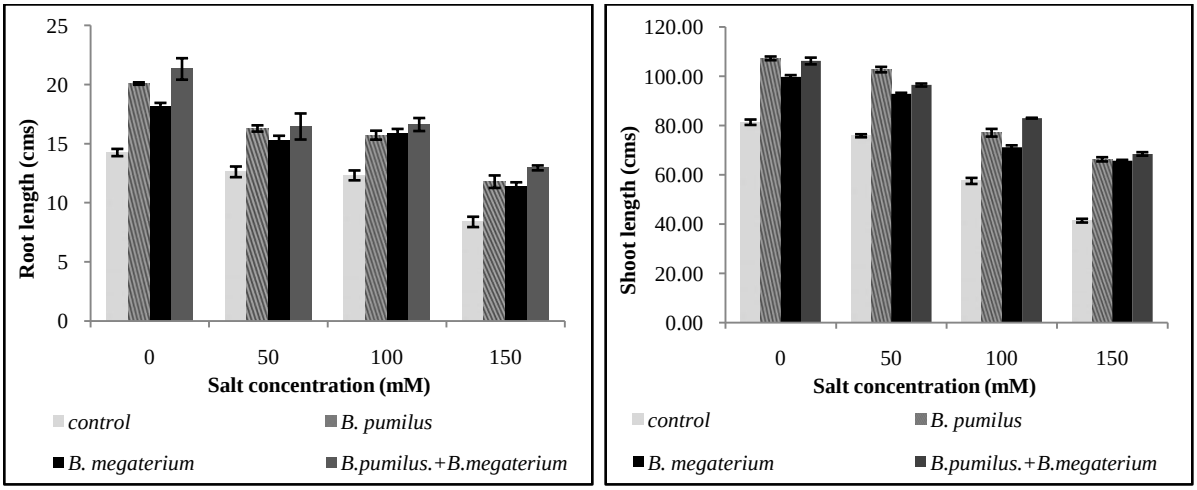

Figure 1 Effect of PGPR on shoot and root length of Pearl millet under NaCl stress

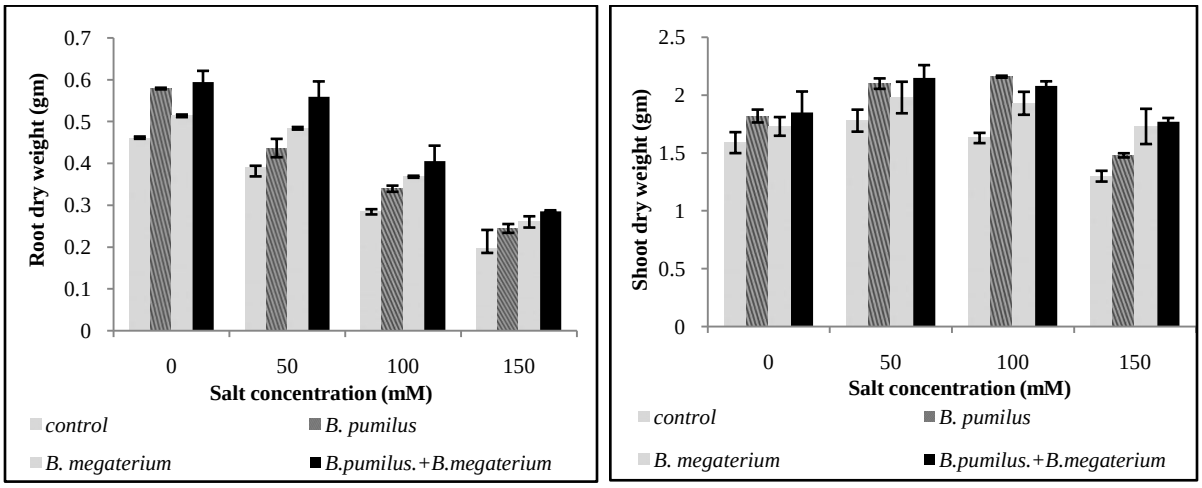

Figure 2 Effect of PGPR on shoot and root dry weight of Pearl millet under NaCl stress

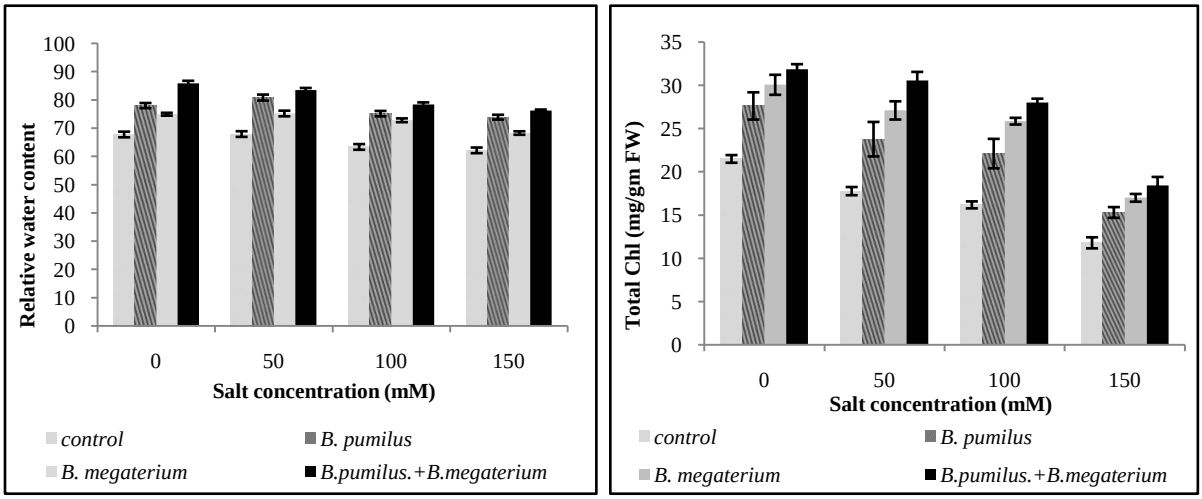

Figure 3 Effect of PGPR on leaf relative water content and total chlorophyll content of Pearl millet under NaCl stress

compared to the untreated control. The total chlorophyll content in pearl millet plants treated with Bp-GN-5 + Bm-JR-12 was found to be increased to 17.3, 24.69, and 45.09 % in comparison to 4.13%, 12.13% and 42.13% in control (only salt) stress treatments respectively (Figure 3).

3.3 Osmolyte Production as Defense Mechanism against Osmotic Stress

An increase in plant biochemical activities was observed under salt stress in pearl millet compared to negative control, i.e., no salt and no PGPR. The treatment of PGPR strains in the salt-amended soil helped increase the biochemical activities more than untreated plants, which in turn helped in plant growth promotion. All the biochemical activities, like the production of phenol content, reducing sugar, amino acid, protein, and proline, were increased in the presence of PGPR strains individually and in combination. However, no significant difference was observed in treated and untreated plants' flavonoid and carbohydrate content production under salt stress (Table 2). Among the treatments, combining two PGPRs, i.e., Bp-GN-5 + Bm-JR-12, showed a significant increase in biochemical activities compared to individual PGPR strain treatment and untreated plants in NaCl stress.

The phenol content in control 0, 50, 100 and 150 mM salt stress plants was found to be 109.43, 124, 97.10 and 169.48 mg/gm DW, respectively, and this improvement was recorded 186.53, 210.60, 237.85 and 323.69mg/gm DW in the salt stress and Bp-GN-5 + Bm-JR-12 combination treated plants, respectively. Similarly, the reducing sugar content in untreated plants was increased in PGPR combination treated plants from 3.17, 7.23, 11.62 and 13.89 mg/gm FW to 6.24, 8.83, 14.8 and 16.7, respectively. Similarly, the amino acid content was 277.71, 316.06, 357.01 and 343.72ug/gm FW in untreated pearl millet plant during salt stress, which was found to be enhanced to 377.64, 404.51, 454.81 and 466.95ug/gm FW in Bp-GN-5 + Bm-JR-12 treated plants. The

Table 2 Effect of PGPR on biochemical activity of Pearl millet under salt stress in pot study

Salt concentration	Treatments	Reducing sugar (mg/gmFW)	Carbohydrate (mg/gm FW)	Protein (mg/gm FW)	Proline (umol/min mg FW)
0 mM	Control	3.17±0.98^{b}	16.37±0.17^{c}	31.47±0.75^{d}	112.33±0.17^{d}
	Bp-Gn-5	5.16±0.88^{a}	18.08±0.38^{b}	38.473±0.63^{c}	123.23±0.27^{b}
	Bm-Jr-12	5.97±0.53^{a}	17.54±0.67^{b}	41.41±0.75^{b}	121.60±0.06^{c}
	Bp-Gn-5 + Bm-Jr-12	6.24±0.45^{a}	19.29±0.31^{a}	48.89±0.51^{a}	130.54±0.07^{a}
50 mM	Control	7.22±0.37^{b}	19.77±0.06^{c}	34.60±0.49^{d}	123.43±0.10^{d}
	Bp-Gn-5	7.52±0.11^{b}	21.29±0.42^{ab}	44.39±0.66^{c}	132.10±0.25^{b}
	Bm-Jr-12	7.84±0.04^{ab}	20.51±0.10^{bc}	49.93±0.59^{b}	129.45±0.25^{c}
	Bp-Gn-5 + Bm-Jr-12	8.82±0.57^{a}	22.33±0.33^{a}	53.44±0.38^{a}	133.36±0.37^{a}
100 mM	Control	11.62±0.40^{a}	21.39±0.09^{d}	35.41±0.28^{d}	140.96±0.44^{d}
	Bp-Gn-5	13.95±1.50^{a}	23.49±0.17^{b}	46.93±0.35^{c}	156.86±1.15^{b}
	Bm-Jr-12	13.70±0.28^{a}	22.58±0.12^{c}	51.21±0.18^{b}	151.96±0.24^{c}
	Bp-Gn-5 + Bm-Jr-12	14.79±0.75^{a}	24.57±0.24^{a}	53.58±0.65^{a}	160.93±0.46^{a}
150 mM	Control	13.89±0.57^{a}	23.54±0.07^{c}	28.50±0.20^{d}	157.99±0.35^{d}
	Bp-Gn-5	15.41±1.44^{a}	25.36±0.34^{b}	48.65±0.61^{c}	171.61±0.59^{b}
	Bm-Jr-12	14.83±0.85^{a}	24.27±0.12^{c}	53.04±0.32^{b}	165.66±0.37^{c}
	Bp-Gn-5 + Bm-Jr-12	16.69±0.29^{a}	26.55±0.12^{a}	58.46±0.13^{a}	187.50±1.16^{a}

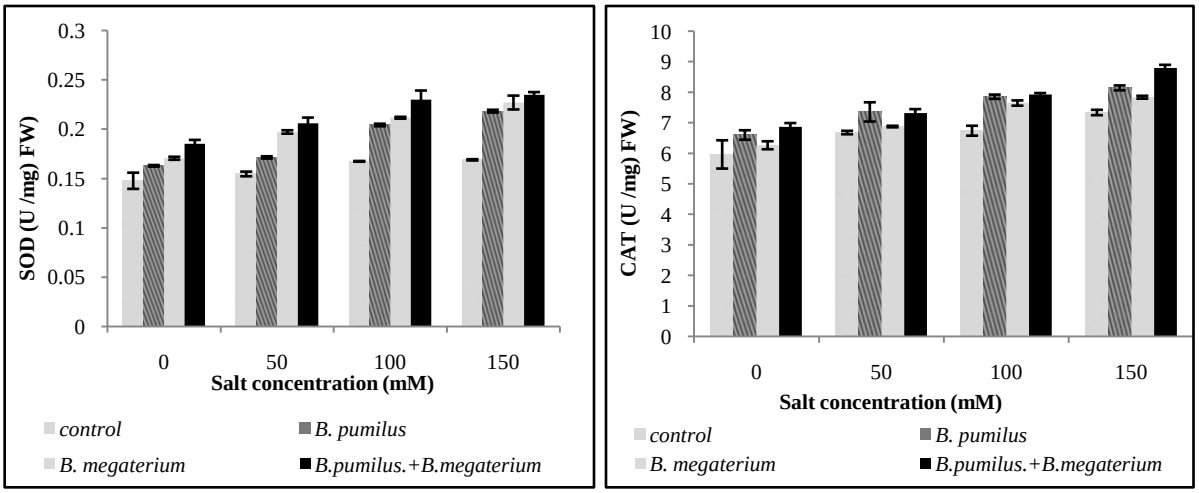

Figure 4 Effect of PGPR on SOD and CAT content of Pearl millet under NaCl stress

protein content in Bp-GN-5 + Bm-JR-12 treated plants were found to be increased to 48.90, 53.45, 53.58 and 58.47 mg/gm FW from 31.50, 34.60, 35.41 and 28.50 in 0, 50, 100 and 150mM salt stress, respectively. Similarly, the proline content in Bp-GN-5 + Bm-JR-12 treated plants were found to be 130.54, 133.36, 160.94 and 187.50 µmol/min mg FW from 112.33, 123.43, 140.96 and 157.99 respectively during salt stress (Table 2). Combining two PGPR strains increased tissue sugar levels and osmolytes to enhance tolerance to short-term salinity stress.

3.4 ROS Scavenging Activity as a Defense Mechanism against Oxidative Stress

The enzymes are responsible for salt tolerance in plants, i.e., superoxide dismutase (SOD) and catalase, were also investigated in the present study and were observed to be enhanced in the PGPR treated pearl millet plants compared to untreated plants under salinity stress. The results obtained for PGR supplementation individually and in combination showed similar

effects on SOD and CAT enzyme production with an increase up to 10-25% and 10-21%, respectively. Similarly, the results obtained for biochemical activities showed the increase in salt concentration induced the plant to produce more phenol, flavonoid, reducing sugar, carbohydrate, amino acid, protein, and proline, which helped the plant to sustain the salt stress.

4 Discussion

The investigated bacterial strains Bp-GN-5 and Bm-JR-12 were isolated from the rhizosphere of mangrove soil. For various microorganisms, mainly bacteria, the rhizosphere region of plants acts as a natural hotspot (reservoir) (Auta et al. 2017; Ling et al. 2022). Saline soils and deep-sea hypersaline sediments, among other environments, have previously been shown to contain Bacillus-like halophilic bacteria. This study was observed to agree with the findings of former studies reported by Sharma et al. 2021, Patel et al. 2023. These strains were investigated for PGP activities viz., IAA production, zinc solubilization, P- solubilization, siderophore and ammonia production. Bp-GN-5 was able to solubilize 35.67 µg/ml phosphate and produce 38.34 µg/ml indole acetic acid, while Bm-JR-12 was able to solubilize 37.67 µg/ml and produce 42.34 µg/ml IAA. Both the isolates were zinc solubilizers, ammonia producers and siderophore producers (Table 1). Exopolysaccharides, siderophores, volatile organic compounds (VOCs), compatible osmolytes, and phytohormones are just a few of the beneficial metabolites produced by ST-PGPR that help the saline-agro ecosystem become more productive (Ullah and Bano 2015; Pallavi et al. 2023). Phosphate solubilizing bacteria have been isolated from soils that have been subjected to environmental extremes, such as saline-alkaline soils, with a high level of nutrient deficit; PSB can solubilize phosphate under moderate saline conditions (Thant et al. 2018; Alotaibi et al. 2022). This increased P content in crops helps mitigate the growth-inhibiting effect of salt stress. PGP bacteria produce IAA, which leads to initiation of rooting, cell division, and expanded root surface range, where root surface region and root engineering play the foremost vital part (Ayaz et al. 2022; Tripathi et al. 2022) on plant development under saline soil conditions. Earlier, Singh et al. (2020) have also reported that multi-trait PGP isolates have shown phosphate solubilization, siderophore, IAA, ammonia and H_2S production, respectively and can be considered as great bioinoculants to alleviate adverse effects of abiotic stresses on plants.

The halotolerant PGPR has been reported to significantly reduce salt stress in agricultural crops. These two Bacilli used in the present study were also investigated for their role in alleviating salinity stress effect on plant growth promotion activities in pearl millet under salt stress. An increase in salt concentration from 0 to 150 mM in soil was found to reduce the growth parameters in pearl millet in the untreated control. Still, the supplementation of the two PGPR strains under salt stress alleviated the negative effect of salinity stress on growth parameters significantly and performed better in plant growth under salinized and non-salinized conditions. The observations made during the study regarding the impact of salt stress, individual PGPR and combinatorial inoculation of both the PGPR, as presented in figures 1, 2, 3 and 4, are in accordance with other previous studies where it has been reported that multi-trait *B. safensis* (BS) and rhizospheric *B. haynesii* (BH) strains showed significant PGP properties under *in vitro* conditions for the growth promotion of the *Amaranthus viridis* plant under salinity (4 dS m^{-1} and 6 dS m^{-1}) conditions. Both strains were effective under abiotic stress conditions such as pH, temperature, salt, and drought (Patel et al. 2023). In the present study, both the bacterial isolates were able to enhance the plant growth under salt stress conditions in comparison to the PGPR untreated control plants, and both *B. megaterium* and *B. pumilus* strains were observed to improve various parameters such as germination percentage, shoot length, root length, FW, DW of pearl millet plant. In another study, *Bacillus* spp. strains, i.e., NMCN1 and LLCG23 isolated from the extreme environments of the Qinghai–Tibetan region of China, showed high salinity stress tolerance and could grow at up to 16% and 18% NaCl concentrations (Venieraki et al. 2021; Ayaz et al. 2022). The variations in germination percentage and all growth parameters could be attributed to the deposition of Na^+ and Cl^- ions in the tissues, eventually compromising the germination metabolism. In another study, Khan et al. (2022) reported that the *Bacillus* strain could improve all growth parameters in wheat plants under 200 mM salt stress conditions.

The PGPR treated plants produce certain osmolytes such as proline, trehalose, and add up to dissolvable sugars that are biosynthesized and accumulate in cytoplasm as consistent solutes in response to osmotic stress under saline conditions. All these are effortlessly retained by plants to control water potential, regulate stomatal openings and transpiration rate, adjust osmosis, and avoid cellular oxidative damage. Several other physiological and biological responses are also controlled, including activating the antioxidant enzyme system, a defence mechanism triggered to eliminate free radicals created under stress and keep their levels low (Habib et al. 2016). This system contains several ROS-scavenging enzymes, including POD, SOD, APX, and CAT, which can reduce abiotic stress, such as salt stress, eliminate free radicals, and prevent the toxicity of ROS produced in stressed cells (Santos et al. 2018). In this work, pearl millet plants inoculated with the isolates Bm JR-12 and Bp GN-5 underwent salinity stress and showed elevated SOD and CAT activity, whereas H_2O_2 and lipid peroxidation decreased. This finding supports that microorganisms positively impact the equilibrium of antioxidant enzymes detoxifying ROS metabolism (Santos et al. 2018). Under conditions of salt stress, ROS, such as SOAs and hydrogen peroxide, are typically formed at high rates and lead to oxidative damage to the cell structure (Sahu et al. 2021).

In this study, it was also observed that the two halotolerant strains *B. megaterium* JR-12 and *B.pumillus* GN-5 consortium treated plant significantly increased protein content in comparison to the uninoculated control in pearl millet under salt stress (150 mM NaCl). The results were consistent with the findings of Ullah and Bano (2015), who discovered that PGPR inoculation to crops enhanced the levels of osmolytes such as proline, sugars, and amino acids compared to un-inoculated controls.

Conclusion

This study's results indicate that PGPR positively impacts the physicochemical properties of inoculated plants, resulting in improved water conditions and increased accumulation of compatible solutes and antioxidant properties. These changes in cellular metabolism ultimately led to improved growth and yield of salt-tolerant and sensitive strains under salt stress. However, salt-tolerant cultivars showed much better growth and yield than susceptible ones. Individual and co-inoculation showed more pronounced effects on pearl millet by playing an essential role in improving growth and yield. The study shall enable these microorganisms to be utilized as biofertilizers for improved pearl millet crop production in saline soils. It can be concluded that the two PGPR strains, *B. pumilus* isolate GN-5 and *B. megaterium* isolate JR-12, can further be used in agricultural practice for alleviating the adverse salinity stress effects on plant growth promotion activities in different crops under salt stress conditions.

Author Contributions

P. and ST conceptualized and designed the experiments. P., ST collected and analyzed the data. P., RKM, NS and ST contributed to the manuscript. AV and ST supervised the work.

Declaration

There are no conflicts of interest among the authors.

Acknowledgments

The authors would like to thank Dr. Sushil Kumar Sharma for guiding the investigation. The authors are also thankful to Dr. A. K. Saxena, Director, ICAR-NBAIM, Mau Nath Bhanjan, Uttar-Pradesh, for providing the lab facility under the AMAAS project. ST acknowledges the Department of Science and Technology-SERB, Government of India research grant ECR/2017/000697 (2018–2021).

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