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Effect of plant growth-promoting rhizobacterial treatment on growth and physiological characteristics of *Triticum aestivum* L. under salt stress

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Abstract

Salinity stress is a serious abiotic stress that affects crop quality and production. Rhizospheric microbes have immense potential in synthesizing and releasing various compounds that regulate plant growth and soil physicochemical properties. The aim of the present study was to evaluate the efficacy of indole-3-acetic acid (IAA)-producing rhizobacteria as biofertilizers under salt stress. Among the isolated strains from various soil samples, *Bacillus megaterium* strain PN89 with multifarious plant growth-promoting traits was selected and used as a monoculture and co-culture with two other standard strains. The plant promoting activity was evaluated using the paper towel method and pot test to observe the effects on the early stage and vegetative growth of wheat (*Triticum aestivum* L.). The treatment using PGPR strain presented noticeable but varying effects on plant growth under salt stress, that is, PGPR treatment often displayed a significant increase in germination percentage, root and shoot length, and other growth parameters of wheat compared to those in the non-inoculated control. Thus, these results suggest that *B. megaterium* PN89 can be applied as a bio-fertilizer to alleviate salt stress in *T. aestivum*.

Keywords: Germination test, IAA production, PGPR, Salinity, *Triticum aestivum* L

Introduction

Soil salinization, one of the most prevalent soil degradation processes, threatens soil fertility [1, 2], and the increase in soil salinity because of less irrigation and drainage can result in poor crop productivity [3]. This is because salinity stress caused by the presence of high levels of salt ions is considered a major abiotic stress to crops [4]. One method to reduce soil salinity-related environmental stress in plants is to use beneficial microorganisms as bio-fertilizers instead of chemical fertilizers or pesticides [5, 6]. The soil area in immediate contact with the root hair is called the rhizosphere, wherein an ingenious trade-off occurs between the plant roots and soil organisms [7]. Because of their extremely low mobility and relatively large size, plant roots obtain a limited amount of nutrients from the soil. However, roots can employ various biological allies to overcome this hurdle. One of the most probable allies is plant growth-promoting rhizobacteria (PGPR), which inhabit the plant rhizosphere or adhere to plant roots [8].

PGPR colonization profoundly alters development and hormone homeostasis in host plants. Plant hormones include auxin, ethylene, cytokinin, gibberellin, abscisic acid, jasmonic acid, and salicylic acid [9], which act as messengers to coordinate the regulation of plant cellular processes, including abiotic stress responses and plant–pathogen interactions [9, 10]. Indole-3-acetic acid (IAA) is a common plant hormone of the auxin class, which is

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also synthesized by PGPR [11]. Auxins are involved in leaf induction, root growth and differentiation. In addition, auxins play an important role in establishing and maintaining the beneficial plant–PGPR interactions [12, 13]. PGPR affects the amount and localization of auxin, in addition to the direction of their movement in plants [14]. Furthermore, PGPR strains positively affect several useful functions such as soil improvement and pest control and promote nutrient absorption and crop growth [15]. In the present study, PGPR strains were isolated and characterized to examine the alleviation of abiotic salt stress in wheat (*Triticum aestivum* L.).

Material and methods

Soil sampling and isolation of bacteria

Soil samples were collected from 72 rhizosphere regions in Korea in April 2017, and were immediately placed in an icebox for transport, and a portion of each sample was stored at 4 °C. Microbes were isolated from soil samples using the streak plate method [16]. Approximately 1 g of rhizospheric soil samples that were tightly adhered to plant roots were transferred into a 15 mL conical tube containing 9 mL deionized water. After vortexing for 10 min, 100 µL of this suspension was transferred into a microtube containing 900 µL of nutrient broth (NB) to obtain a 10^{-1} dilution. After additional serial dilutions (10^{-3}, 10^{-4}, and 10^{-5}), the diluents were plated on nutrient agar and incubated at 37 °C for 24 h. Nutrient Broth No. 2 (Oxoid, United Kingdom) was used in this study.

Measurement of IAA production

The IAA produced was measured by inoculating the rhizobacterial isolates in 3 mL Luria–Bertani broth (LB; DB Difco, NJ, USA) supplemented with L-tryptophan (100 µg/mL). Bacterial cultures were grown for 72 h with shaking (120 rpm) at 28 °C, and 1 mL of this broth was transferred into a microtube and centrifuged at 9,500 × *g* for 2 min. The supernatant (100 µL) was transferred into 96-well microplate and mixed with Salkowski's reagent (1 mL of 0.5 M $FeCl_3$ in 49 mL of 35% $HClO_4$) in a 1:2 ratio. After incubating in dark for 30 min at room temperature, the color intensity was measured at 530 nm using a microplate reader [17].

Detection of siderophore production

Siderophore production was determined using the universal chrome azurol S (CAS) method [18]. Single colonies of bacterial isolates were inoculated into 3 mL LB broth and incubated overnight with shaking (200 rpm) at 37 °C, and 40 µL of this broth was dropped at the center of CAS agar plate containing the blue indicator dye, hexadecyltrimethylammonium (HDTMA). The siderophore developed by the formation of a ternary complex CAS/Fe(III)/HDTMA was indicated by orange halos around the bacterial colonies. After incubating for 5 days at 28 °C, the formation of the orange zone surrounding the colonies was considered positive for siderophore production.

Detection of phosphate solubilization

Single colonies of bacterial isolates were inoculated into 3 mL NB broth and incubated overnight with shaking (200 rpm) at 37 °C. For each strain, 40 µL of this culture was dropped at the center of the Pikovskaya agar plate [19]. After incubating for 5 days at 28 °C, the development of clear zones around the colonies was regarded as positive for phosphate solubilization.

Detection of hydrogen cyanide production

Bacteria were streaked on a modified agar plate, wherein NB was amended with 4.4 g glycine/L. Whatman filter paper no. 1, which was soaked in a solution containing 2% sodium carbonate in 0.5% picric acid, was placed on top of the plate. Plates were sealed air-tight with parafilm and incubated at 28 °C for 4 days. A color change in the filter paper from orange to reddish-brown color was considered positive for hydrogen cyanide (HCN) production [20].

Detection of ammonia production

Isolates were grown in 10 mL peptone broth at 30 °C for 48 h. After centrifugation, the bacterial supernatant was mixed with Nessler's reagent [21]. The change in color from brown to yellow was indicative of ammonia production.

Detection of cellulase activity

The cellulase activity was screened by spot inoculation of bacterial isolates on carboxymethylcellulose (CMC) agar medium [22]. The plates were incubated at 30 °C for 48 h. Congo red solution was poured onto the CMC plates to observe the clear zones around the bacterial colonies. Isolates showing clear zones were considered positive for cellulase activity.

Detection of protease activity

Extracellular protease production was screened using 1% skim milk agar. The bacterial isolates were spotted onto the surface of skim milk agar plates. Protease production was determined by the formation of a clear zone surrounding the bacterial isolates due to the breakdown of the protein. That is, colonies surrounded by clear zones were considered positive for protease activity [23].

Phylogenetic analysis

Genomic DNA of the bacterial isolate was extracted using the GeneAll Exgene DNA extraction kit (Seoul, Korea) according to the manufacturer's instructions. Genomic DNA was PCR-amplified using the 16S universal primer pairs 27F (5-AGAGTTTGATCCTGGCTCAG-3) and 1492R (5-GGTTACCTTGTTACGACTT-3). The obtained PCR products were purified and sequenced by Macrogen (Seoul, Korea). To obtain 16S rDNA sequences, the sequences were aligned using the BioEdit program (http://labtools.us) and analyzed using the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Seed inoculation

Seeds were surface-sterilized with sodium hypochlorite for 20 min and then rinsed five times with autoclaved distilled water. Wheat seeds were pre-soaked in autoclaved distilled water and stored at 4 °C for 3 days. Monoculture and co-culture of *Paenibacillus pabuli* strain P7S (PP7S; GenBank: KF010780.1), *Pseudomonas nitroreducens* strain IHB B 13,561 (PnIHB; GenBank: KJ767371.1; accession number KCCM1133P by the Korean Culture Center of Microorganisms), and isolated strain (*Bacillus megaterium strain* PN89; PN89 hereafter) in the present study were grown in LB broth with shaking (200 rpm) at 37 °C for 18 h. For the co-culture experiment, the bacterial suspension was adjusted to 1×10^7 CFU/mL using a UV spectrophotometer (optical density at 600 nm). The inoculum levels were calibrated using a turbidity of 0.4 McFarland Standard (approximately 5×10^7 CFU/mL) (https://en.wikipedia.org/wiki/McFarland_standards). Then, the seeds were mixed with bacterial suspension at 140 rpm in a 50 mL flask for 2 h at room temperature.

Germination tests

The seeds were separated from the bacterial suspension using sterile sieves and placed on a double layer of sterile filter paper (Whatman no. 1) within a Petri dish (90 mm × 15 mm) at a density of 15 seeds. The sterile filter paper was moistened with 10 mL sterile distilled water. Then, the plate with seeds was placed within a plant growth chamber at 23 °C in dark. Germinated seeds were counted every 24 h for 5 days, and seeds with a radicle of a minimum of 2 mm were considered germinated. Germination percentage (GP) and germination rate index (GRI) were obtained after 120 h, and the GP was calculated using the following formula: GP = (total number of germinated seeds/total number of seeds) × 100. The GRI is the percentage of germination per day and was calculated using the following formula: GRI = (total number of seeds/total number of days) [24]. The final seedling lengths and weights were measured and compared. The vigor index was calculated using the following formula: Vigor index = (mean root length + mean shoot length) × % germination [25]. The plants were dried to a constant weight at 60 °C for 48 h in an incubator (BI-600 M; Jeio Tech, Daejeon, Korea). Germination tests were performed according to the regulations of the International Seed Testing Association (ISTA; http://www.seedtest.org/en/home.html) [26].

Salt stress on seed germination

Plant seeds were surface-disinfected by immersing in 70% ethanol for 1 min, and then washed thrice with sterile distilled water. Thereafter, 15 seeds were placed in each Petri dish lined with two layers of filter paper wetted with 5 mL of 0, 50, 100, 150, 200, 250, and 300 mM sterile NaCl solution. All Petri dishes treated with NaCl solutions of varying concentrations were incubated in a plant growth chamber at 23 °C in dark. Germination was monitored for 96 h. The number of germinated seeds was recorded every 24 h.

Pot tests

Seeds of *T. aestivum* were sterilized with sodium hypochlorite for 20 min and then rinsed five times with sterile distilled water. After 3 days of storage in sterile distilled water at 4 °C, the seeds were cultured on MS (Murashige and Skoog) agar plates containing 2% sucrose in a growth chamber at 25 ± 2 °C under a 16 h photoperiod using cool white fluorescent tube lights, while humidity was maintained at > 60%. After 3 days of cultivation, uniform seedlings were selected and randomly divided into seven sets of four replicates. One set was used as the untreated control, and the remaining six sets were used for treatment; all seven sets were transferred to a pot. Culture broth (1 mL, 1×10^8 CFU) was spread on and around each seed for monoculture and co-culture treatments. Three seedlings (one replicate) were sown in pots filled with sterile Sunshine® Mix #4 (Sun Gro Horticulture, MA, USA). Seedlings were watered with 50 mL of sterile distilled water or 200 mM NaCl solution every two days. Each growth parameter was measured after 20 days.

Data analysis

SPSS 20 software package (IBM, IL, USA) was used to analyze the data. The standard error (SE) of all experiments was performed in triplicate and represented in the table (value ± SE) and figures (error bar). Statistical significance was determined using analysis of variance (ANOVA) and Duncan's test using means comparison with a probability level of 0.05.

Results and discussion

Among the identified rhizobacterial isolates, six were selected according to their plant growth-promoting (PGP) characteristics. The seven PGP characteristics shown in Additional file 1: Table S1 were suggested as beneficial characteristics of PGPR strains [8]. Six PGPR isolates produced a relatively high level of IAA in the range of 32.25–91.26 µg/mL. The other six PGP characteristics were siderophore production, phosphate solubilization, protease production, HCN production, ammonia production, and cellulase production (Additional file 1: Table S1). Siderophore-producing PGPR increased Fe (III) ion supply to plants in the rhizosphere and is, therefore, known to enhance plant growth and crop productivity. Furthermore, Fe (III) ions are chelated to form Fe (III)–siderophore complexes, inhibiting the activity of competitive microbes [7]. In the present study, the selected isolates differed in their siderophore production abilities; PN89 exhibited the highest siderophore-producing ability among the isolates (Additional file 1: Table S1, Fig. 1). Phosphorous is the second most important nutrient required for plant growth [27]. Phosphate-solubilizing bacteria convert insoluble phosphate to soluble monobasic and dibasic phosphate ions, which are available for plant uptake through the roots [28]. The ability to produce a clear zone around the bacterial colony implies that the bacteria can solubilize mineral phosphorus in the rhizosphere. All the selected rhizobacterial strains exhibited similar phosphate solubilization activity (Additional file 1: Table S1, Fig. 1). Proteases are mainly involved in the hydrolysis of peptide bonds [23]. All the selected bacterial isolates presented a similar extent of protease production (Additional file 1: Table S1, Fig. 1). HCN produced by rhizobacteria plays a critical role in the biological control of pathogens [29]. In the present study, all the selected rhizobacterial strains displayed similar extent of cyanogenic activity, indicating that the selected bacterial strains facilitated plant antibiosis to a certain extent (Additional file 1: Table S1). A similar extent of ammonia production was observed in the six selected bacterial isolates (Additional file 1: Table S1). Cellulase activity causes pathogen suppression through degradation of the cellulosic cell walls of plant pathogens [30]. Similar extent of cellulase activity was evident in the six selected bacterial isolates (Additional file 1: Table S1).

Numerous genera, including *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, and *Rhizobium*, have been found in the plant rhizosphere [31]. The six selected strains were identified by phylogenetic analysis of the 16S rRNA gene sequencing data. This sequence was used for a BLAST search performed on the NCBI database. The five isolated strains PN89, PN92, PN94, PN95, and PN99 were identified as *Bacillus* strains with 99% similarity (Additional file 1: Table S2). In contrast, the PN32 strain was identified as a *Lysinibacillus* sp. with 99% similarity. *Bacillus* sp. represents a significant fraction of soil microbial communities and is widely used in agriculture to promote plant growth and protect against plant pathogens. In particular, *Bacillus megaterium* and *Bacillus aryabhattai* are the representative rhizobacteria that solubilize soil phosphorus, enhance nitrogen fixation, and produce siderophores [32, 33].

Seed germination tests of *T. aestivum* were performed with the six selected strains using the paper towel method (Fig. 2). Seeds of *T. aestivum* treated with the PN89 strain displayed a higher average shoot length than that of the other strains (Fig. 2A). The shoot dry weight of PN89 was the highest among all the tested strains (Fig. 2B). No

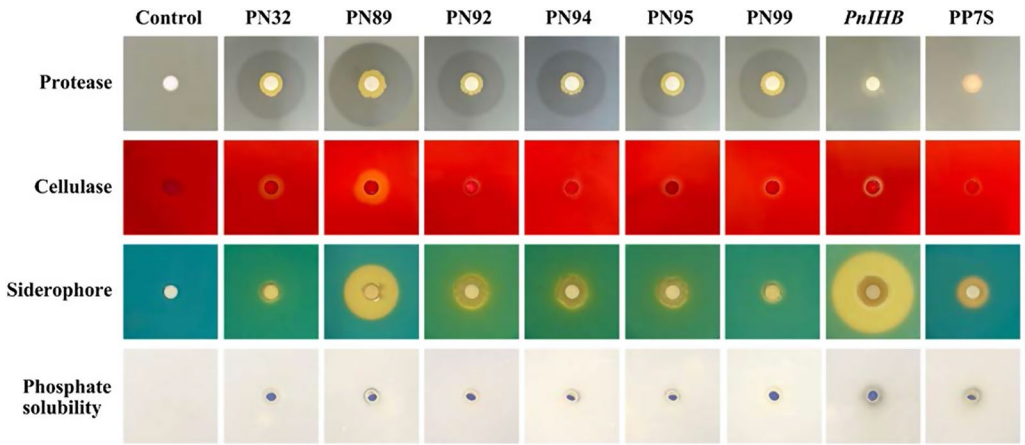

Fig. 1 Photographs of selected bacterial strains exhibiting various plant growth-promoting (PGP) properties. Tests for protease, cellulase, siderophore production, and phosphate solubility were performed. For relative comparison of each property, refer to the Additional file 1: Table S1

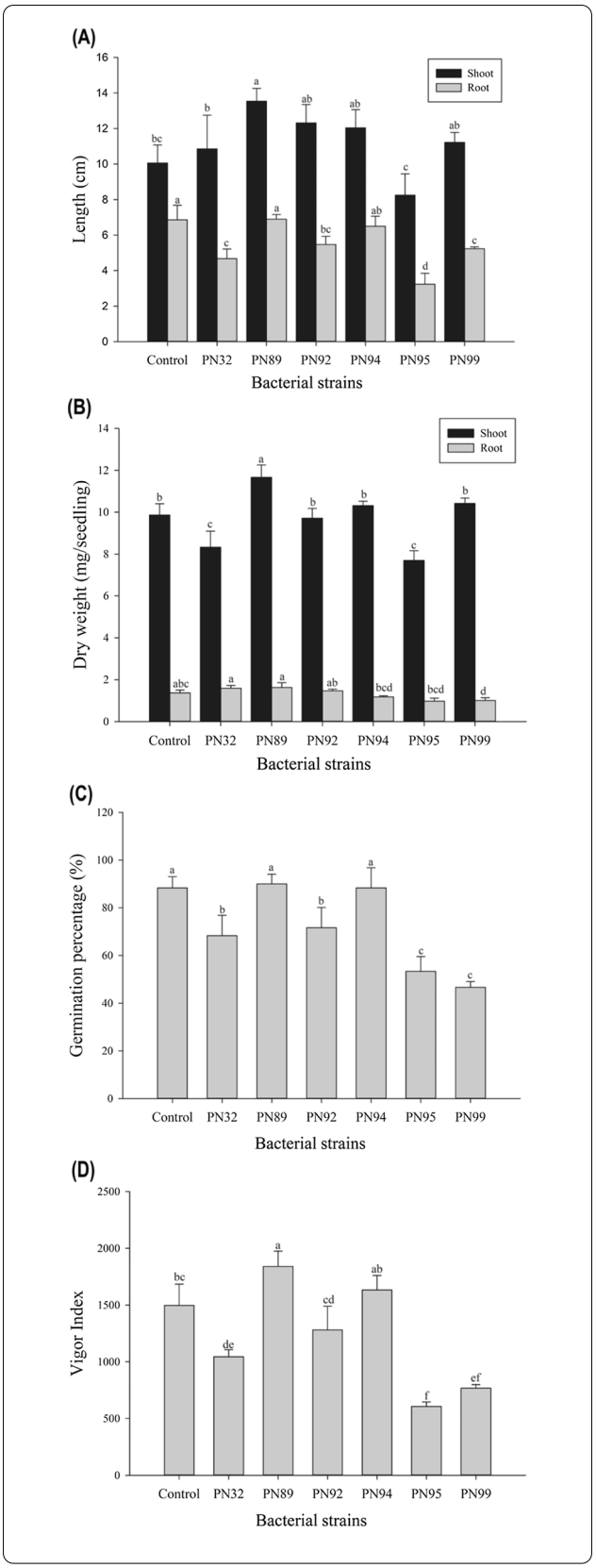

Fig. 2 Plant growth parameters of *T. aestivum* seedlings for different bacterial strains. **A** Final shoot and root length. **B** Final shoot and root dry weight. **C** Germination percentage (GP). **D** Vigor index (VI). Control had no bacterial treatment. Error bars represent standard deviation (n = 3). Different superscripted letters for each shoot/root and GP/VI values indicate significant difference among various bacterial cultures according to Duncan's test ($p = 0.05$)

specific trend was observed for root length and root dry weight among the tested strains. The GP was similar and highest with the control, PN89, and PN94 (Fig. 2C). The vigor index of PN89 was the highest among the tested strains (Fig. 2D). Overall, PN89 presented the maximum beneficial effect on *T. aestivum* seed germination. Therefore, *B. megaterium* strain PN89 was selected to examine early seed germination and for pot trials under salt stress.

The extent to which plant seeds withstand germination under salinity could be affected by the existence of rhizobacterial strains [34]. Thus, rhizobacterial strains were selected to observe the beneficial effects of different degrees of salinity on seed germination. The germination extent of *T. aestivum* seeds was examined at a salinity range of 0–300 mM. The GP decreased with increasing NaCl concentration (Fig. 3). The GPs were 93%, 86%, 60%, 36%, and 16% for 0, 50, 100, 150, and 200 mM, respectively. However, no germination was observed at NaCl concentrations of 250 and 300 mM. These results showed that the seed GP was affected by the salt concentration. Therefore, salt concentrations of 0, 100, 150, and 200 mM were selected for subsequent experiments.

Two additional strains, PP7S and PnIHB, were included for comparison because they are known to enhance plant growth through different mechanisms; PP7S contributes to anthocyanin accumulation, and the PnIHB strain enhances nitrate uptake [35, 36]. Seed GP and other growth parameters of *T. aestivum* were also observed by treating with co-cultures of PN89, PP7S, and PnIHB. Thus, a total of eight different treatments were considered for this experiment: control (uninoculated), PN89, PnIHB, PP7S, M1 (co-cultures of PN89 + PnIHB), M2 (co-cultures of PnIHB + PP7S), M3 (co-cultures of PP7S + PN89), and Mix (co-cultures of PN89 + PnIHB + PP7S). The usefulness of co-culture inoculants over monoculture inoculants has been reported previously, wherein use of co-culture inoculants enhances plant productivity through diverse mechanisms [37].

Germination and early seedling growth responses of wheat under salinity conditions were assessed using a paper towel method. The GP was considerably affected by each treatment (Fig. 4A). The GP at 0 mM was similar among control, PN89, and PP7S but lower for PnIHB. For control and PM89, GP at 100 and 150 mM was in the

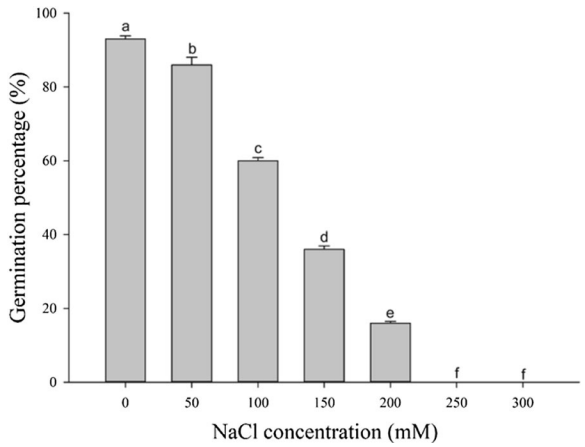

Fig. 3 Seed germination percentages of *T. aestivum* under different salinity levels after cultivation for 120 h. Within each germination percentage, mean values followed by the same lowercase alphabet are not significantly different ($p = 0.05$)

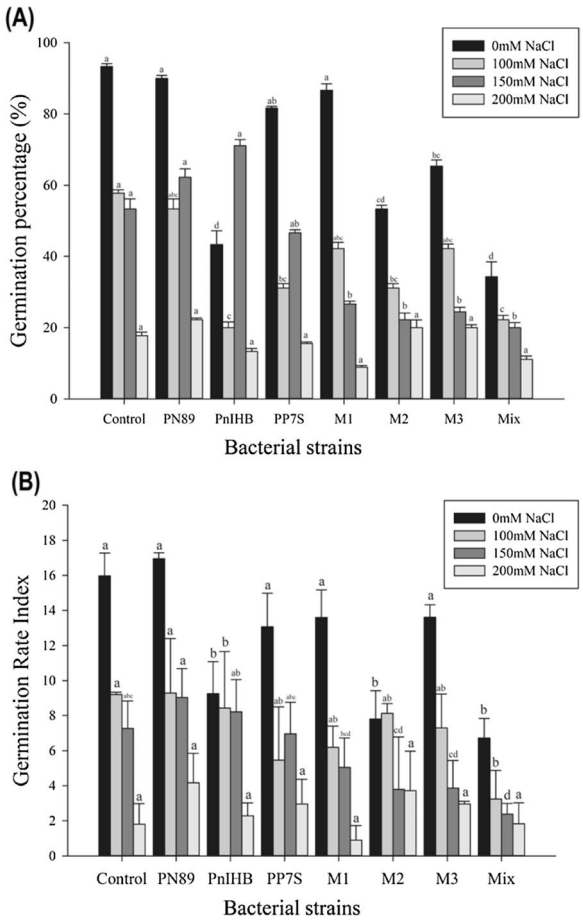

Fig. 4 **A** Germination percentage and **B** germination rate index of *T. aestivum* seedlings upon treatment with various rhizobacterial inoculants under salt stress. Control had no bacterial treatment. M1, co-cultures of PN89 + PnIHB; M2, co-cultures of PnIHB + PP7S; M3, co-culturing of PP7S + PN89; Mix, co-cultures of PN89 + PnIHB + PP7S. Error bars represent standard deviation (n = 3). Different superscripted letters for each NaCl concentration indicate significant difference among various bacterial cultures according to Duncan's test ($p = 0.05$)

range of 53–62%, and the GP was similar for the same salt concentration. In contrast, the GPs at 100 and 150 mM were approximately 20 and 71%, respectively, for PnIHB. GPs at 100 and 150 mM for PP7S were approximately 31 and 47%, respectively. Furthermore, GP was expected to decrease with increasing salt concentration, but GPs were higher at 150 mM NaCl than at 100 mM NaCl for PnIHB and PP7S. The overall GP at 0 mM was lower for co-culture treatment than for monoculture treatment, decreasing in the order of M1 > M3 > M2 > Mix. In particular, M1 at 0 mM had 85% GP, which was similar to that at 0 mM NaCl of control. Mix treatment at 0 mM exhibited 37% GP, which was the lowest among all treatments with 0 mM NaCl. For the co-culture treatment, the average GP gradually decreased with increasing concentrations of NaCl. The GP at 200 mM was in the range of 9–22% (Fig. 4A).

The GRI is the percentage of germination per day during the germination period. Higher GRI values indicate higher and faster germination rates [24]. The GRI was considerably affected by each treatment (Fig. 4B). GRI at 0 mM was similar among control, PN89, and PP7S but substantially lower for PnIHB. GRI at 100 and 150 mM was in range of 7.27–9.21 for control, PM89, and PnIHB. GRI at 100 and 150 mM was in the range of 5.46–6.96 for PP7S, a value slightly lower than that of the aforementioned three treatments. The overall GRI at 0, 100, 150, and 200 mM for co-culture treatment decreased with increasing NaCl concentration (Fig. 4B). In particular, GRI values at 0 mM for M1 and M3 were similar to that of control.

The shoot length at 0 mM was similar among control, PN89, PnIHB, and PP7S and in the range of 8.7–10.1 cm/seedling. The shoot length at 100 mM was slightly higher for control and PN89 than for PnIHB and PP7S. The shoot length at 150 mM was considerably higher for PN89 and PnIHB than for control and PP7S (Fig. 5A). The shoot lengths of M1, M2, M3, and Mix were similar or slightly lower than that of the control. For the co-culture treatment, shoot length decreased with increasing NaCl concentrations. Shoot lengths at 100 and 150 mM were slightly or substantially lower for co-culture than for monoculture treatment. For co-culture treatment, the overall shoot length at 200 mM was relatively lower than that for monoculture treatment (Fig. 5A). For control, root length was not significantly

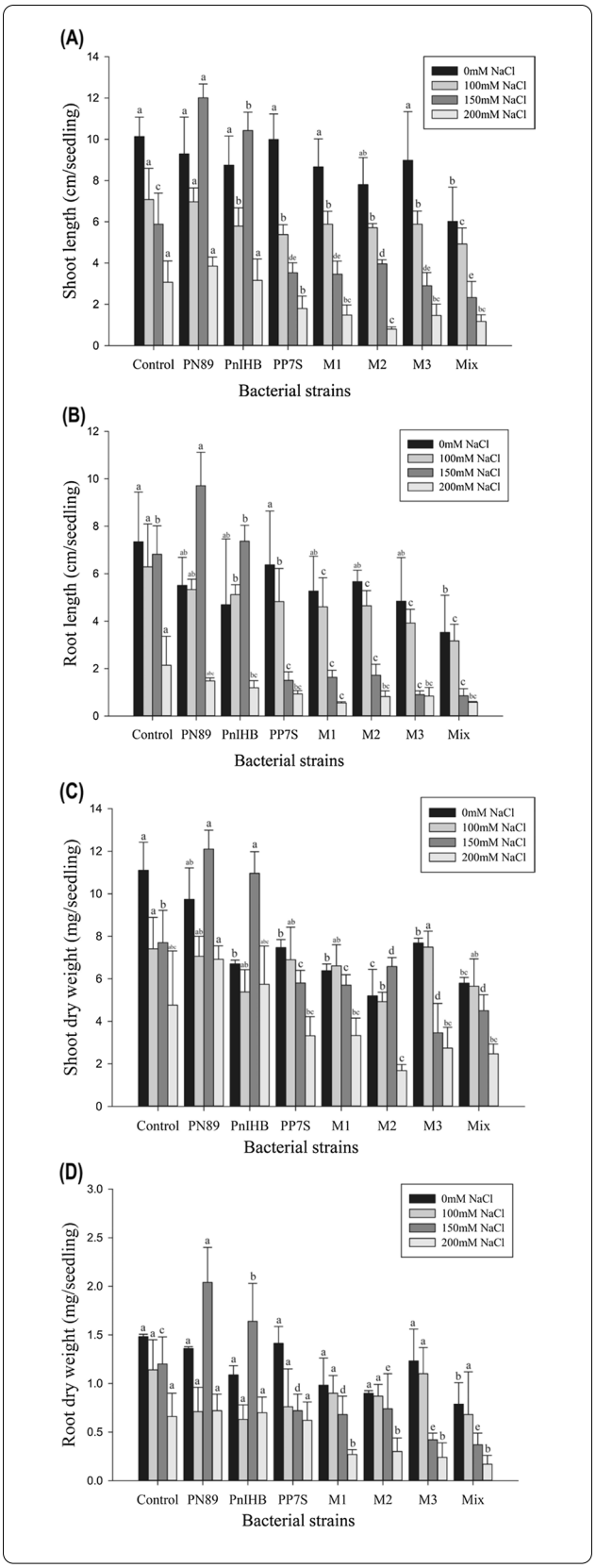

Fig. 5 **A** Shoot length, **B** root length, **C** shoot dry weight, **D** root dry weight of *T. aestivum* seedlings upon treatment with various rhizobacterial inoculants under salt stress. Control had no bacterial treatment. M1, co-cultures of PN89 + PnIHB; M2, co-cultures of PnIHB + PP7S; M3, co-cultures of PP7S + PN89; Mix, co-cultures of PN89 + PnIHB + PP7S. Error bars represent standard deviation (n = 3). Different superscripted letters for each NaCl concentration indicate significant difference among various bacterial cultures according to Duncan's test ($p = 0.05$)

different between 100 and 150 mM NaCl and was in the range of 6.3–6.8 cm/seedling (Fig. 5B). For PP7S, the root length gradually decreased with increasing NaCl concentrations. The average root lengths at 150 mM were higher with PN89 and PnIHB than with control and PP7S and in the range of 7.3–9.7 cm/seedling. The root lengths for M1, M2, and M3 were similar to those for PP7S. The average root lengths of Mix were slightly lower than those for M1, M2, and M3, but the difference was not significant (Fig. 5B). Shoot dry weight shared a similar trend with shoot length for the control and three monoculture treatments. Shoot dry weights of M1, M2, and Mix were in the range of 4.5–6.6 mg/seedling. The average shoot dry weights of 100 mM for the control and M3 were slightly higher than those for M1, M2, and Mix (Fig. 5C). The overall trend of root dry weight was similar to that of shoot dry weight (Fig. 5D). In particular, root dry weights for PN89 and PnIHB were higher at 150 mM than for the other monoculture treatments. All these results suggest that, with a few exceptions, salinity adversely affected germination and initial growth of *T. aestivum* in monoculture and co-culture treatments (Figs. 4 and 5).

Without salt treatment, the effect of the selected PGPR strains on actual plants was determined by conducting an in vivo pot test. *T. aestivum* seeds were grown in sterile soil for 20 days, and the growth parameters were measured (Fig. 6). The results showed that inoculating the selected PGPR endowed a positive effect on *T. aestivum* growth compared to the controls treated with only distilled water. The fresh weight of stem was in the range of 0.35–0.58 g/plant, among which M1 (co-cultures of PN89 + PnIHB) presented the highest value of 0.58 g/plant. Fresh weight of root was in the range of 0.25–0.35 g/plant, among which M3 (co-cultures of PP7S + PN89) displayed the highest value of 0.35 g/plant (Fig. 6A). The dry weight of the stem was in the range of 0.040–0.062 g/plant, among which M1 and M2 had the highest average value of 0.062 g/plant. Average dry weights among co-culture treatments were in the order M1 ≈ M2 > M3. The dry weight of roots was in the range of 0.011–0.021 g/plant and no specific

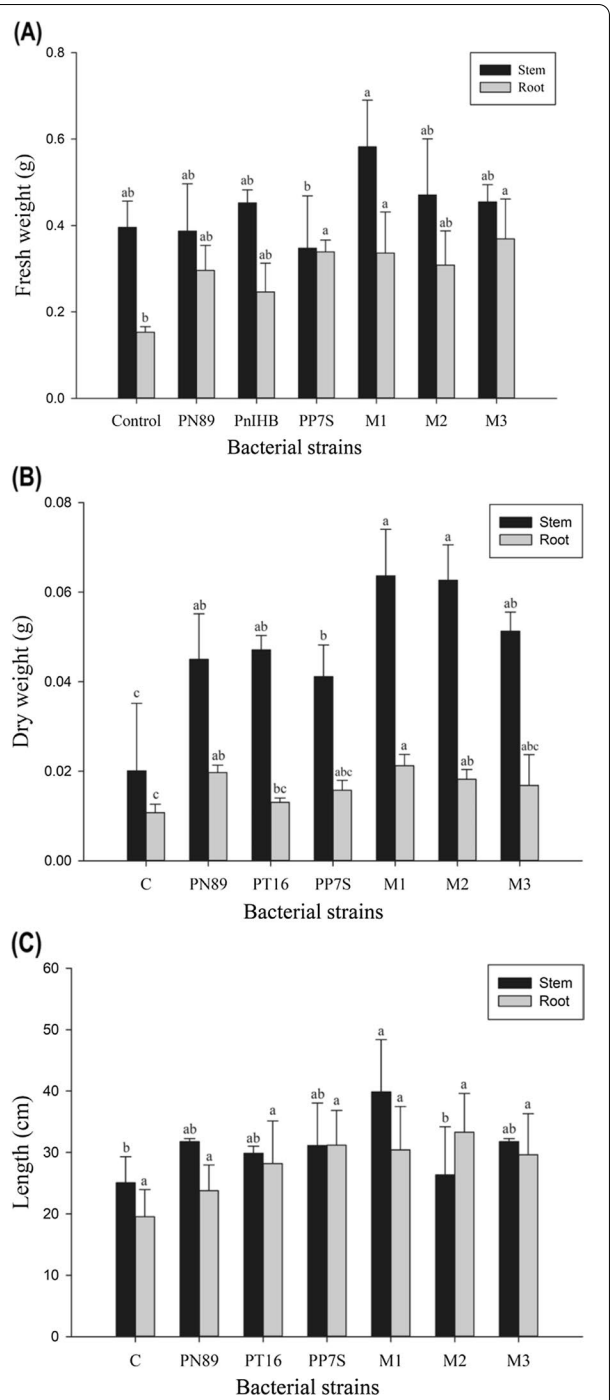

Fig. 6 Growth parameters of **A** fresh weight, **B** dry weight, **C** length during pot tests with *T. aestivum* upon treatment with various rhizobacterial inoculants. Control had no bacterial treatment. M1, co-cultures of PN89 + PnIHB; M2, co-cultures of PnIHB + PP7S; M3, co-cultures of PP7S + PN89. Error bars represent standard deviation (n = 3). Different superscripted letters for each stem and root values indicate significant difference among various bacterial cultures according to Duncan's test ($p = 0.05$)

trend was found within all treatments (Fig. 6B). No specific trend was found for stem length, but M1 had the highest average stem length. The average stem length was in the range of 27.1–41.0 cm, among which M1 had the highest value of 41.0 cm. No specific trend was observed for root length. (Fig. 6C).

Under salt stress conditions, at 200 mM NaCl, both the control and PGPR-treated seedlings exhibited an overall lower fresh weight, dry weight, and length of stem and root when compared with those without salt treatment (Fig. 7). The fresh weights of control under salt treatment were 0.80 g and 0.60 g for stem and root, respectively. Upon treatment with PGPR, the fresh weight of the stem was in the range of 1.2–1.5 g, which was higher than that of the control. The average root weights were greater for PN89, PP7S, and M1 than for the control (Fig. 7A). The average dry weights of stems were in the range of 0.018–0.030 g and considerably greater for PN89, PT16, M1, and M3 than that for the control. The average root lengths were greater for PN89, M1, and M3 than that of control (Fig. 7B). The average lengths of the PGPR-treated plants were greater than those for the control plants. However, the stem and root lengths were not significantly different from those of the control, except for the stem of PN89 and root of M1 (Fig. 7C). All results in Fig. 7 showed that PGPR treatment considerably affected the growth under salt stress conditions. The newly isolated strain PN89, as a bio-fertilizer, considerably protected *T. aestivum* during the initial growth compared to those previously reported PT16 and PP7S [35, 36]. In particular, mixture of two strains, including M1, often enhanced the weight and length of stems and roots of *T. aestivum*.

The present experiments were performed under sterile conditions. In addition, PGPR in the field coexists with many other soil microbes. To make further approach to in situ condition with isolated strains, the future study need to be performed under sterile or non-sterile condition with concomitant application of several isolated strains while excluding standard strains such as PnIHB and PP7S. The present experiments suggest that the selected PGPR strain without and with standard strains (i.e., symbiosis) can supply a tool or method under salt stress for field application in the growth of *T. aestivum* with further development. Considering the scenarios of serious environmental damage and food security, uncovering different unknown PGPR could be an effective approach to produce high crop yields in the future.

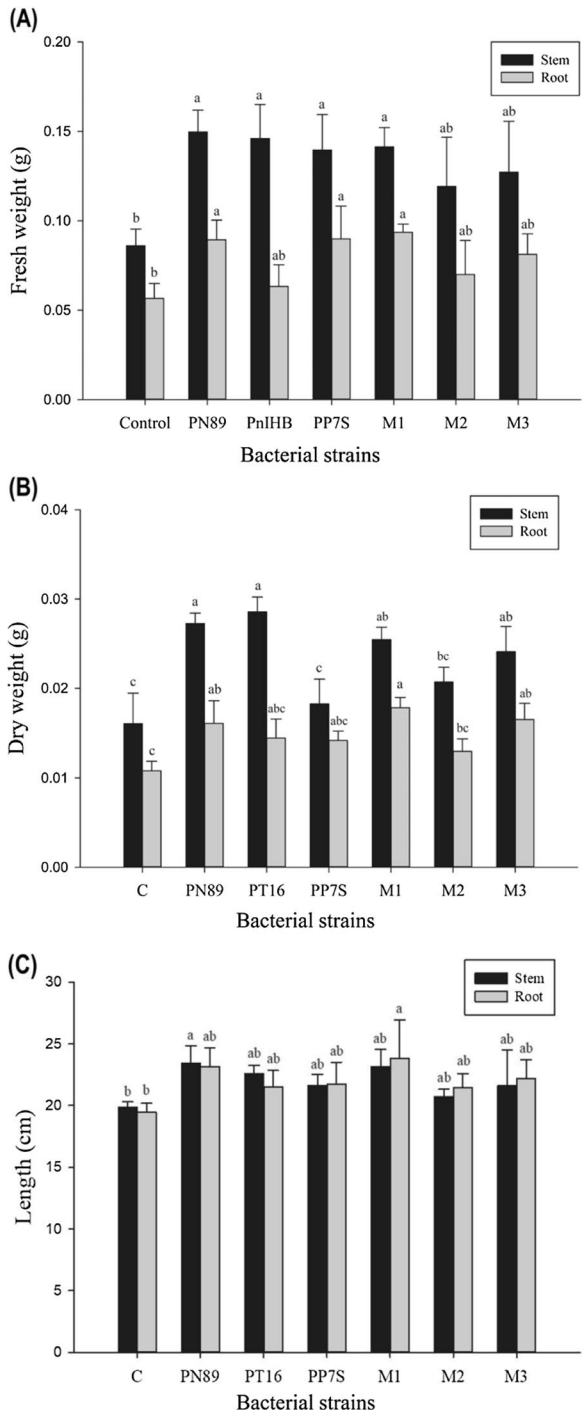

Fig. 7 Growth parameters of **A** fresh weight, **B** dry weight, **C** length during pot tests with *T. aestivum* upon treatment with various rhizobacterial inoculants under salt stress. Control had no bacterial treatment. M1, co-cultures of PN89 + PnIHB; M2, co-cultures of PnIHB + PP7S; M3, co-cultures of PP7S + PN89; Mix, co-cultures of PN89 + PnIHB + PP7S. Error bars represent SD (n = 3). Different superscripted letters for each stem and root values indicate significant difference among bacterial cultures according to Duncan's test ($p = 0.05$)

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-021-00663-w.

Additional file 1: Table S1. Characterization of selected bacterial strains by various plant growth-promoting (PGP) properties. **Table S2**. Molecular analysis of selected bacterial isolates based on 16S rDNA sequencing.

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Authors' contributions
DGL wrote and revised the manuscript. JML performed the experiments and wrote the manuscript. CGC performed the experiments and interpreted the data. NC, HL, and JCM supervised the study and interpreted the data. All authors have read and approved the final manuscript.

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Availability of data and materials
Not applicable.

Declarations

Competing interests
The authors declare no conflict of interest.

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