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Exploring indigenous *Bacillus* spp. as a biostimulant to enhance the growth and yield of rice under glasshouse conditions

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Abstract. *Shultana R, Zuan ATK, Rana MM, Naher UA, Paul PLC, Akter M, Shupta SA, Roy TK. 2025. Exploring indigenous* Bacillus *spp. as a biostimulant to enhance the growth and yield of rice under glasshouse conditions. Asian J Agric 9: 131-139.* Plant growth-promoting rhizobacteria (PGPR) appeared as an emerging approach to sustainable agriculture because of their ability to reduce the need for synthetic fertilizers. This study evaluated five potential *Bacillus* strains isolated from rice-growing areas in northern Malaysia for their ability to enhance rice growth parameters under laboratory and glasshouse conditions. Among the five strains, two UPMRB9 (*Bacillus tequilensis* 10b) and UPMRE6 (*Bacillus aryabhattai* B8W22) exhibited all the desired traits. These strains were subsequently inoculated into three potential rice plants, resulting in improved morphological (plant height, leaf area), physiological (photosynthesis, transpiration, stomatal conductance, dry biomass, and grain yield), and biochemical (total chlorophyll, total proline content, total soluble sugar, and uptake of N, P, K, Ca, and Mg) attributes. Inoculation of Putra-1 plants with UPMRB9 showed a 10.76% increase in plant height, a 50.82% increase in grain yield, a 12.32% increase in colonization, and a 56% increase in stomatal conductance. Additionally, inoculation of BRRI dhan67 with UPMRB9 reduced the transpiration rate by 100.89%. MR297 plants treated with UPMRB9 demonstrated an 86.58% increase in leaf area, a 125.81% increase in photosynthesis, a 222.19% increase in dry biomass, a 56.03% increase in total chlorophyll, a 62.93% increase in proline content, and a 62.93% increase in total soluble sugar (TSS). Therefore, UPMRB9, as a multiple plant growth promoter, could be an alternative to synthetic fertilizers for rice cultivation.

Keywords: *Bacillus*, biofertilizer, nutrient uptake, PGPR, proline

INTRODUCTION

Rice (*Oryza sativa* L.) is a crucial dietary staple worldwide, providing sustenance and nutrition to billions. Estimates indicate that global rice production reached 517 million tons in 2023 (FAO 2023). Rice feeds more than half of the worldwide population, making a significant energy contribution to low-income families as well. In tropical areas, rice is commonly grown as a staple crop (Abbas et al. 2019). The utilization of cultivars and management techniques like fertilization have a major impact on rice yield. Many nations' contemporary agricultural production systems mainly depend on synthetic fertilizers and pesticides. However, worries over the harmful effects on human health and the environment have been raised by the extensive usage of these synthetic agrochemicals. Furthermore, over-reliance on synthetic fertilizers may increase the demand for inputs to sustain current yield levels. Natural ecosystems, on the other hand, provide nutritional advantages, insect pest resistance (Roy et al. 2024a), and more sustained yields. Overuse of synthetic fertilizers may change the physical, chemical, and biological properties of the soil. Using beneficial microbes that enhance crop growth is a sustainable substitute (Ríos-Ruiz et al. 2020). Furthermore, rice is widely distributed across different soils and climates globally in the face of changing climate scenarios, so it has significant strategic value (Fahad et al. 2019; Ríos-Ruiz et al. 2020). But, over the past few decades, the use of beneficial soil microorganisms, such as Plant Growth-Promoting Rhizobacteria (PGPR), for sustainable agriculture has expanded significantly due to the negative environmental impact and rising prices of synthetic fertilizers (Ikhajiagbe and Osazee 2019). *Bacillus* spp., commonly found in soil, serves as PGPR by forming robust endospores that resist high temperatures and chemical concentrations (Kashyap et al. 2019). *Bacillus* spp. promote plant growth by improving seed emergence, increasing biomass, and enhancing grain yield (Egamberdieva et al. 2017; Weemstra et al. 2022). PGPR inoculants offer a natural remedy by facilitating advantageous biological processes in agricultural ecosystems with little adverse effects. As a result, there is increasing interest in creating and utilization of biofertilizers for different crops based on PGPR.

The production of Auxin (especially indole-3-acetic acid, IAA) for root development, nutrient uptake facilitation, atmospheric nitrogen fixation (Mashabela et al. 2022), siderophore production for iron uptake (Singh et al. 2022), phosphorus solubilization (Dhole et al. 2023), ACC deaminase synthesis (which cleaves ethylene, essential in response to stress), and biofilm production (which improves bacterial adhesion to root tissue and nutrient uptake) are some of the mechanisms by which PGPR promotes plant growth. The PGPR consortia are typically used as biofertilizers since individual strains frequently lack the necessary mechanisms to support host growth (Redondo-Gómez et al. 2023). Still, there is limited research for analyzing how PGPR affects rice growth (Shultana et al. 2020).

Several previous studies have demonstrated that mung bean inoculation with the rhizobacterial strain *Bacillus drentensis* significantly increased stomatal conductance, transpiration rate, and total chlorophyll content in comparison to the uninoculated control (Mahmood et al. 2016). According to a previous study, the inoculation of holy basil plants with *Pseudomonas* sp. and *Bacillus lentus* effectively reduced the effects of salinity by increasing the levels of photosynthesis, minerals, and the antioxidant system (Golpayegani 2011). Proline and total soluble sugar concentration are sensitive physiological indexes that act as osmolytes to shield plant growth from oxidative stress. Plants may resist osmotic and salt strains by controlling proline, which stabilizes proteins and membranes, scavenges free radicals, and buffers cellular redox potential in salt stress (Hayat et al. 2012). Higher total soluble sugar (TSS) content in plants also serves as a defense mechanism against salinity. An increase in proline and TSS in wheat plants is a significant PGPR contribution to osmotolerance (Upadhyay et al. 2012). Therefore, it was hypothesized that the inoculation of the indigenous PGPR strain, *Bacillus* spp., could increase the growth and yield of rice. In this study, efforts were made to explore the ability of potential bacterial isolates to promote rice growth through enhancing physiological characteristics and the regulation of biochemical metabolites and their influence on rice yield under glasshouse conditions.

MATERIALS AND METHODS

Plant growth-promoting traits of the selected strains (in vitro screening)

The five bacterial strains were previously isolated and identified as salt-tolerant strains (Shultana et al. 2020). Among them, two potential strains, UPMRB9 (*Bacillus tequilensis* 10b) (accession number: NR 104919.1) and UPMRE6 (*Bacillus aryabhattai* B8W22) (accession number: NR 115953.1), were further inoculated into three rice varieties to observe their effects on growth and yield. The Indole-3-acetic acid (IAA) production was quantified using the colorimetric method of Gordon and Weber (1951) with some amendments of salinity concentrations. The selected isolate was cultured in tryptic soy broth (TSB) modified at different concentrations of NaCl (0, 0.5, 1, 1.5, and 2 M) and supplemented with 5 mL of L-tryptophan which acts as the precursor of IAA, and allowed shaking for 24 h. After incubation, approximately 1.5 mL of the culture was transferred into sterile Eppendorf tubes and centrifuged at 7000 rpm for 7 min. The IAA production was then measured by adding 2 mL of Salkowski reagent to the bacterial culture supernatant, and the absorbance was recorded at 535 nm using a spectrophotometer. The ability of the bacteria to fix atmospheric nitrogen, phosphate and potassium solubilization ability and siderophore production was estimated following the method of (Tan et al. 2014).

The plant inoculation study under glasshouse conditions

The soil samples were collected from Tanjong Karang, Selangor, Malaysia, allowed to dry, and mixed well. The soil series is bernam; pH 4.23. The available nutrients of the initial soil were 0.62% of N, 0.08 mg/kg of P, 0.29 mg/kg of K, 0.23 mg/kg of Ca and 0.33 mg/kg of Mg. Each plastic pail, with dimensions of 30 cm in diameter and 46 cm in height, was filled with 20 kg of soil. Following the fertilizer recommendations of the Department of Agriculture, Kuala Selangor, Malaysia, urea, triple super phosphate (TSP), and muriate of potash (MOP) were applied @170, 80, and 150 kg ha^{-1}, respectively. Urea fertilizer was used in three equal splits at 15, 45, and 60 days after transplanting (DAT), while TSP and MOP fertilizers were applied as basal doses. The treatments were: A. Rice variety: i. BRRI dhan67; ii. Putra-1; and iii. MR297; B. PGPR strains: i. No inoculum; ii. UPMRB9; and iii. UPMRE6. The seeds of three rice varieties, namely BRRI dhan67, Putra-1, and MR297, were initially submerged in ethanol (95%) for 10 s. After removing the ethanol, seeds were submerged again in 3% NaOCl for 1 min (ChloroxTM) followed six times by rinse with sterilized dH_2O water. The glass Petri dishes covered with moistened filter papers (3 layers) were used to germinate 20 surface-sterilized seeds. To keep the filter paper moist (Whatman No 1), sterile dH_2O was added regularly and the seed germination percentage was recorded daily for up to 4 days. After 5 days, the seedlings were transplanted. Twenty surface-sterilized seeds were germinated on three layers of moistened filter papers (Whatman No. 1) with distilled water added regularly to keep the papers moist. After five days, the germinated seeds were transplanted. The fully grown selected bacterial strains were cultured in Tryptic Soy Broth (TSB) medium and shaken for 24 hours before their cells were collected through centrifugation. Bacterial cells (approximately 10^8-10^9 CFU mL^{-1}) and sterile distilled water (as a control) were inoculated onto five-day-old rice seedlings and kept to settle for one hour. A single seedling of each rice cultivar was transplanted into the soil of each plastic pail. The roots of each rice plant were re-inoculated at 14 days after transplanting (DAT), with sterile distilled water added as an uninoculated control. The leaf area was measured at the maximum tillering stage, calculated following the formula stated by Shultana et al. (2013). The photosynthesis, transpiration, and stomatal conductance rates data were estimated at the flowering stage for each rice variety using the LI-COR (LI-6400XT) portable photosynthesis system (LI-COR Inc., Lincoln, Nebraska, USA). Mature leaves were randomly selected, plucked, sealed in plastic bags, and transported to the laboratory. For additional biochemical analysis, the leaves were crushed with liquid nitrogen, wrapped in plastic bags, and kept in a freezer at -80°C.

The content of proline in leaves was measured by considering the protocol as stated by Bates et al. (1973) and Jin et al. (2019) with a slight adjustment of chemical concentrations. An amount of 0.1 g of fresh leaves was homogenized with 2 mL of 5% (w/v) sulfosalicylic acid and was allowed for centrifugation (Sigma 3K30) at 10,000 rpm for 10 minutes. One milliliter of supernatant was taken in a test tube holding 1 mL of glacial acetic acid and 1 mL acid ninhydrin (including the standard). The test tubes were kept in a water bath (95°C) for 1 hour following cool in an ice bath. Toluene (2 mL) was mixed on each tube including the standard. The proline concentration was determined by taking the absorbance reading at 520 nm using the microplate reader (BioTek 800 TS).

The total soluble sugar was determined by adopting the method of Yemm and Willis (1954) and Johnson et al. (2023). Leaf samples (100 mg) were homogenized in 10 mL of 80% ethanol following centrifugation (Sigma 3K30) at 4,000 rpm for 20 min. After the collection of supernatants, the residue was re-submerged with 10 mL of 80% ethanol following centrifugation at 4,000 rpm for 20 min. Then the supernatant was collected again. The two times collected supernatant was mixed with a known amount of ethanol extract (0.1 to 0.2 mL). Further, the test tubes were placed in a water bath at 100°C to make it dry through evaporation. Subsequently, the test tubes were allowed to cool in room temperature (27°C). Afterward, in each test tube, 1mL of distilled water was added and thoroughly mixed. Anthrone reagent (4 mL) was added to each test tube along the wall of the test tube and mixed gently. The test tube was heated again in a water bath at 100°C for 10 min and rapidly cooled on an ice bath. The absorbance of the prepared sample was measured by spectrophotometer (UV-3101PC, Shimadzu) at 620 nm against a reagent blank.

The plant tissue samples were oven-dried for a full 72 hours at 70°C. Using a plant grinder, the samples were grounded, and standard nutrient analysis procedures were used to measure the amounts of the nutrients (Awang et al. 2009). N, P, K, Ca, and Mg concentrations were measured using an Atomic Absorption Spectrophotometer (AAS) (Perkin-Elmer 400). By multiplying the concentration values by the plant dry weight, the uptake of N, P, K, Ca, and Mg was calculated (Goteti et al. 2013).

Yield data components, including filled grains, 1000-grain weight, and grain yield, were recorded after the terminal harvest. After the grains acquired a moisture content of 14% through air drying, they were weighed and counted (Tan et al. 2014).

Statistical analysis

Using SAS 9.4 software, the recorded data were subjected to Analysis of Variance (ANOVA). Tukey's Studentized Range (HSD) test compared means at a 0.05 probability level. Correlation coefficient analysis was performed using R Studio (version 2023.12.1) and the "metan" package.

RESULTS AND DISCUSSION

Screening of selected bacterial isolates for plant growth-promoting traits

The plant growth-promoting (PGP) traits of the five selected bacterial isolates varied widely. UPMRB9 exhibited significantly higher IAA production (16.33 μg mL^{-1}), followed by UPMRE6 and UPMRG1. UPMRE6 was recorded to solubilize the highest amount of phosphorous resulting in the highest concentrations of available phosphorus (41.50 μg mL^{-1}) followed by UPMRB9 and UPMRE3 while the highest available potassium concentration (15.79 mg L^{-1}) was measured by UPMRE6. High atmospheric nitrogen fixation and siderophore production were observed in strains, UPMRB9 and UPMRE6 (Table 1).

Effect of bacterial inoculation on the morphological characters of three rice cultivars

Bacterial inoculations significantly increased the plant height of the tested rice varieties. The rice variety, Putra-1 and MR297 responded positively with UPMRB9. However, the inoculation of Putra-1 showed the greatest increase in plant height (117.55 cm), followed by UPMRE6 (111.50 cm) which is a 10.76% and 5.06% increase compared to the uninoculated plant. BRRI dhan67 equally responded to UPMRB9 and UPMRE6 inoculations. However, regardless of rice variety, the lowest plant height was measured without bacterial inoculation (Figure 1.A). The UPMRB9 inoculations enlarged the leaf areas of the Putra-1 and MR297 rice varieties (482.23 cm). Further, the leaf areas of BRRI dhan67 were increased by UPMRE6 inoculation. The highest significant value was recorded by the inoculations of UPMRB9 to MR297 rice plant (482.23 cm) which is an 86.58% increase over the uninoculated plant (Figure 1.B).

Table 1. Plant growth-promoting traits of different bacterial strains

Bacteria strains	IAA (μg mL^{-1})	Available P (μg mL^{-1})	Available K (mg L^{-1})	N fixation	Siderophore production
UPMRA4	7.00b	22.11c	5.72de	-	-
UPMRB9	16.33a	40.49a	13.82b	++	++
UPMRE3	7.13b	40.14a	6.90cd	+	+
UPMRE6	13.06a	41.50a	15.79a	++	++
UPMRG1	12.49a	35.53b	7.76c	+	+

Note: Means having the same letter (capital) in a column does not differ significantly at alpha 0.05 level by Tukey (HSD)

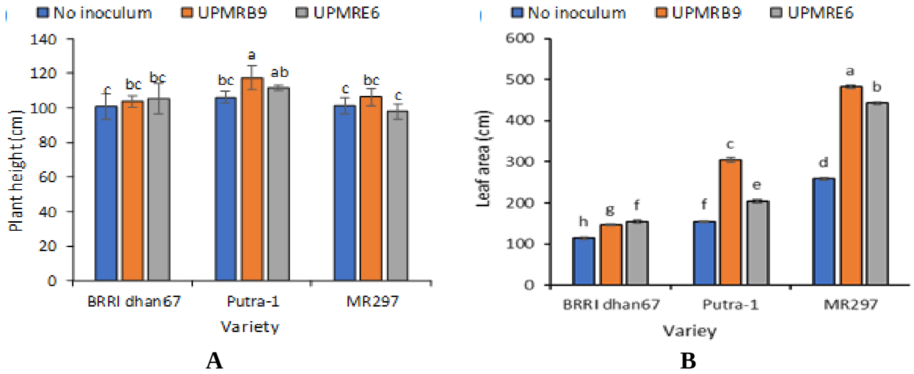

Figure 1. Effect of UPMRB9 and UPMRE6 inoculation on: A. plant height and B. leaf area of tested rice plants. Means having the same letter in each variety does not differ significantly using the Tukey test at $p>0.05$

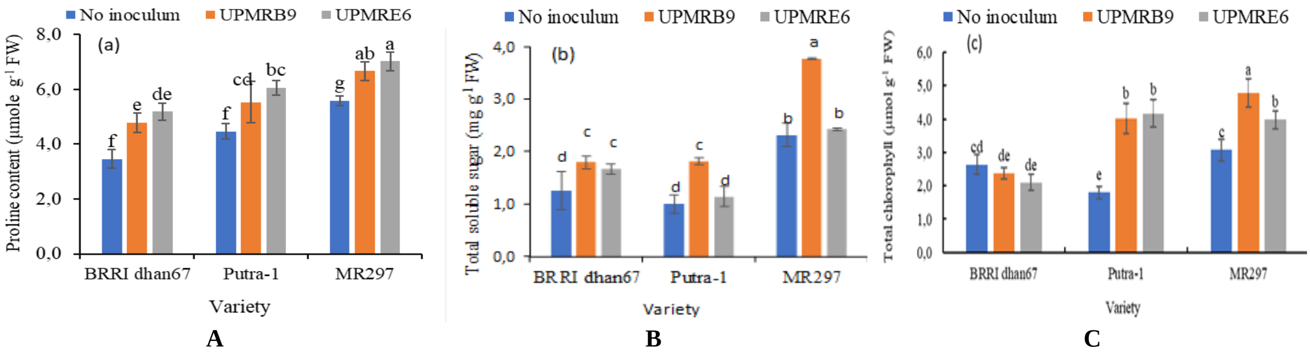

Figure 2. Effect of UPMRB9 and UPMRE6 inoculation on: A. The tested rice plants' proline; B. Total soluble sugar; and C. Total chlorophyll content. Means having the same letter in each variety does not differ significantly using the Tukey test at $p>0.05$

Effect of bacterial inoculations on biochemical properties of three rice plant

The biochemical properties of the three rice varieties followed similar trends with bacterial inoculations. The highest proline regulation was observed in the MR297 variety inoculated with UPMRE6 (7.02 µmole g^{-1} FW), followed by UPMRB9 (6.66 µmole g^{-1} FW) which is 25.81 and 19.35% increase over uninoculated plant. Besides, the lowest proline regulation was observed in BRRI dhan67 regardless of bacterial strains (Figure 2.A). Similarly, the highest total soluble sugar content was found in the MR297 variety inoculated with UPMRB9 (3.78 mg g^{-1} FW) which is 62.93% higher than the uninoculated plant (Figure 2.B). The total soluble sugar was found highest treated with UPMB9 in all three varieties. A similar trend was observed for total chlorophyll content, with MR297 rice variety inoculated with UPMRB9 (4.79 µmole g^{-1} FW), a 56.03% increase compared to the uninoculated plant. Besides, the lowest total chlorophyll content was measured in BRRI dhan67 regardless of bacterial strains (Figure 2.C).

Effect of bacterial inoculations on physiological traits of the tested rice plants

The physiological properties of the tested rice cultivars significantly varied with bacterial inoculations. Regardless of the type of rice, the photosynthesis rate increased dramatically with the inoculation of UPMRB9. However, the highest rate of photosynthesis was measured in the MR297 variety inoculated with UPMRB9 (12.70 µmol CO_2 m^{-2} s^{-1}) which is a 28.41% increase over the UPMRE6 inoculated MR297 rice variety (Figure 3.A). Additionally, enhanced stomatal conductance was recorded in the Putra-1 variety inoculated with UPMRB9 (0.39 mmole m^{-2} s^{-1}) which is 56% of the increase over the uninoculated plant (Figure 3.B). An accelerated rate of transpiration was estimated in BRRI dhan67 without bacterial amendment (4.50 mmole m^{-2} s^{-1}) (Figure 3.C).

Effect of bacterial inoculations on dry biomass and grain yield of the tested rice plants

The dry biomass and grain yield of the tested rice cultivars were significantly enhanced with the assimilation of the tested bacterial strains. The MR297 rice variety accumulated the highest dry biomass with UPMRB9 inoculation (10.31 g) (Figure 4.A). The strain, UPMRB9 inoculation significantly improved the grain yield of three rice varieties**.** However, the highest grain yield was measured in Putra-1 rice inoculated with UPMRB9 (43.21 g/plants) which is a 50.82% increase over the uninoculated plant. Besides, the lowest grain yield was recorded in BRRI dhan67 without bacterial inoculation (23.15 g/plant) (Figure 4.B).

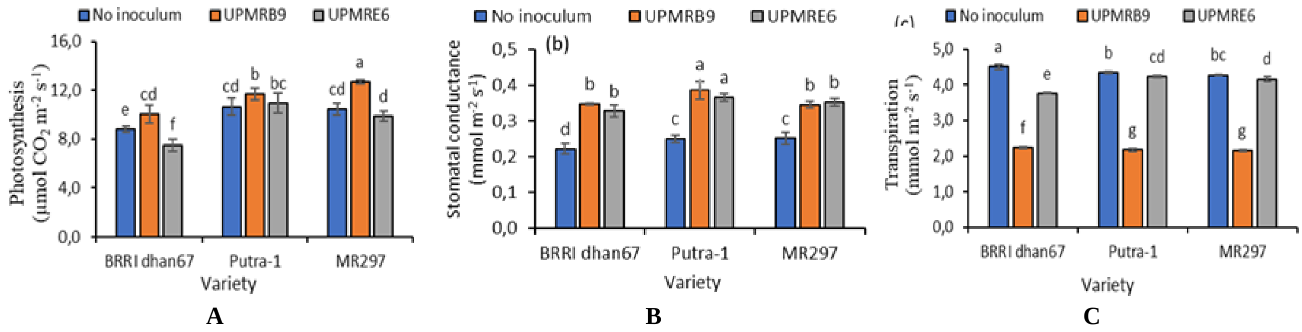

Figure 3. Effect of UPMRB9 and UPMRE6 inoculation on: A. Photosynthesis; B. Stomatal conductance; and C. Transpiration of the tested rice plants. Means having the same letter in each variety does not differ significantly using the Tukey test at p>0.05

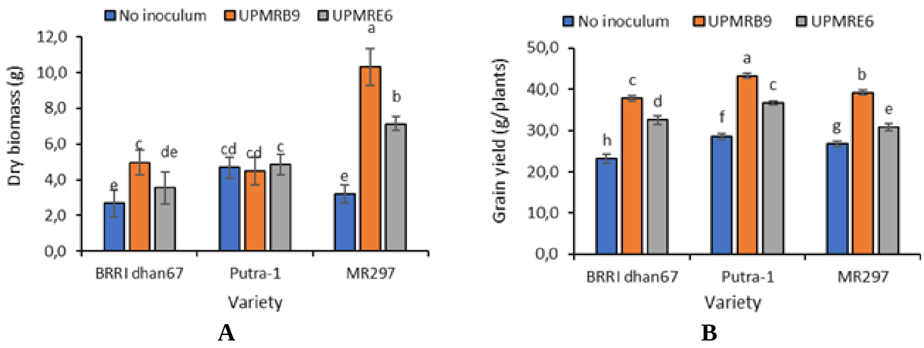

Figure 4. Effect of UPMRB9 and UPMRE6 inoculation on: A. The tested rice plants' dry biomass and B. grain yield. Means having the same letter in each variety does not differ significantly using the Tukey test at p>0.05

The total bacterial population in the rhizosphere of tested rice plants

Bacterial colonization differed among the rice varieties. UPMRB9 showed higher abundance regardless of the rice variety. However, the highest abundance of UPMRB9 was counted in Putra-1 rice (7.20). Besides, the lowest abundance of UPMRB9 was counted in the MR297 rice variety (6.59). The colonization pattern of UPMRE6 was slightly higher with BRRI dhan67 and was not markedly varied between Putra-1 and MR297 (Figure 5).

The uptake of nutrients in PGPR-inoculated rice plants

Bacterial inoculation significantly upgraded nutrient uptake in the rice plants. UPMRE6 inoculation in the Putra-1 variety triggered the uptake of N (3.93 g $plant^{-1}$), P (7.18 g $plant^{-1}$), Ca (5.62 g $plant^{-1}$), and Mg (3.73 g $plant^{-1}$), while UPMRB9 stimulated higher uptake of K uptake in BRRI dhan67 (32.84 g $plant^{-1}$). The increments in N, P, Ca, and Mg content in the Putra-1 variety were 112.43, 152.82, 195.79, and 74.30%, respectively, while K content in BRRI dhan67 increased by 135.08% (Figures 6.A-E).

Correlation between yield and other traits among the three rice varieties

There were various associations observed between PGPR inoculation and rice varieties (Figure 7). Positive and significant correlations were found between grain yield and CFU g^{-1} dry soil, plant height, photosynthesis, stomatal conductance, transpiration, proline content, dry weight and leaf area. However, negative and non-significant correlations were observed with tiller number and total soluble sugar content by UPMRB9 inoculation (Figure 7.A). In UPMRB9 inoculation, significant positive correlations were found between grain yield and plant height, photosynthesis, stomatal conductance, and transpiration, but negative and non-significant correlations with tiller number (Figure 7.B). Without inoculation, positive and significant associations were found between grain yield, plant height and photosynthesis, while negative and significant associations were observed with CFU g^{-1} dry soil, tiller number, and soluble sugar content. Negative and non-significant associations were found with proline content, dry weight, and leaf area (Figure 7.C).

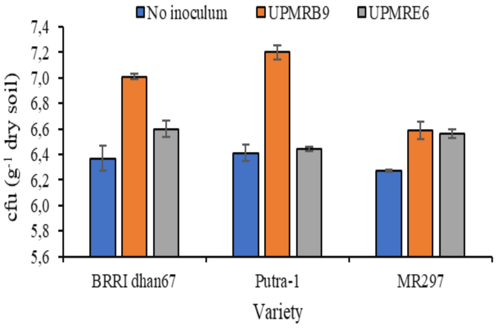

Figure 5. The total population of UPMRB9 and UPMRE6 in the rhizosphere of the tested rice plants. Means having the same letter in each variety does not differ significantly using the Tukey test at p>0.05

Discussion

It has been widely accepted that *Bacillus* spp. could promote plant growth through biological nitrogen fixation, phosphate solubilization, ACC deaminase activity, and the synthesis of siderophores and phytohormones (de Souza et al. 2015). In our study, two bacterial strains, *Bacillus tequilensis* (UPMRB9) and *Bacillus aryabhattai* (UPMRE6) confirmed higher IAA production, increased phosphate and potassium availability, nitrogen fixation, and siderophore production. Earlier, Bahadir et al. (2018) identified four *Bacillus* strains that exhibited multiple plant growth-promoting characteristics. Further, Abdullahi et al. (2022) isolated and identified three *Bacillus* strains (*Bacillus subtilis*, *B. niacini*, and *B. cereus*) from the rice rhizosphere, which exhibited various plant growth-promoting properties. These potential strains are reported to produce ammonia which acts as a source of nitrogen to the plant. Besides, the phosphate solubilizing ability of the strains solubilizes the insoluble form of phosphate into soluble form and makes it available to the plants. Moreover, the indole 3 acetic acid (IAA) is one of the prominent features of plant growth-promoting rhizobacteria which manipulates the growth of their host through cell elongation and root-shoot development.

The vegetative growth phase is critical for any crop, characterized by increased plant height, a greater number of tillers and leaves, and higher root and shoot dry biomass. In this study, *Bacillus tequilensis* inoculation in Putra-1 rice significantly accelerated root growth and stem elongation. Additionally, this inoculation greatly enhanced the leaf area in the MR297 variety. Putra-1 rice, a tall-statured plant, benefited from the high IAA production of the bacterial strain and promotes robust growth of roots. Furthermore, the strain's ability to fix atmospheric nitrogen and solubilize phosphorus and potassium contributed to the extensive leaf area in MR297. Earlier reports have also indicated that bacterial inoculation increased plant height, leaf number, tiller number, root dry mass and shoot dry mass (Sharma et al. 2014). The two *Bacillus* strains significantly stimulated total soluble sugar and proline content in the tested rice cultivars, indicating their capability to induce plant resistance to various stresses. Previous reports suggest that *Bacillus* species, major inhabitants of the rhizosphere and epiphytic microorganisms, exhibit plant growth-promoting activities and stress tolerance (Aswathy et al. 2017). Similarly, under nutrient-scarce conditions, *Bacillus amyloliquefaciens* SN13 and its consortium enhanced proline and total soluble sugar content in rice (*Oryza sativa* L. var. IR-36) (Bisht et al. 2020).

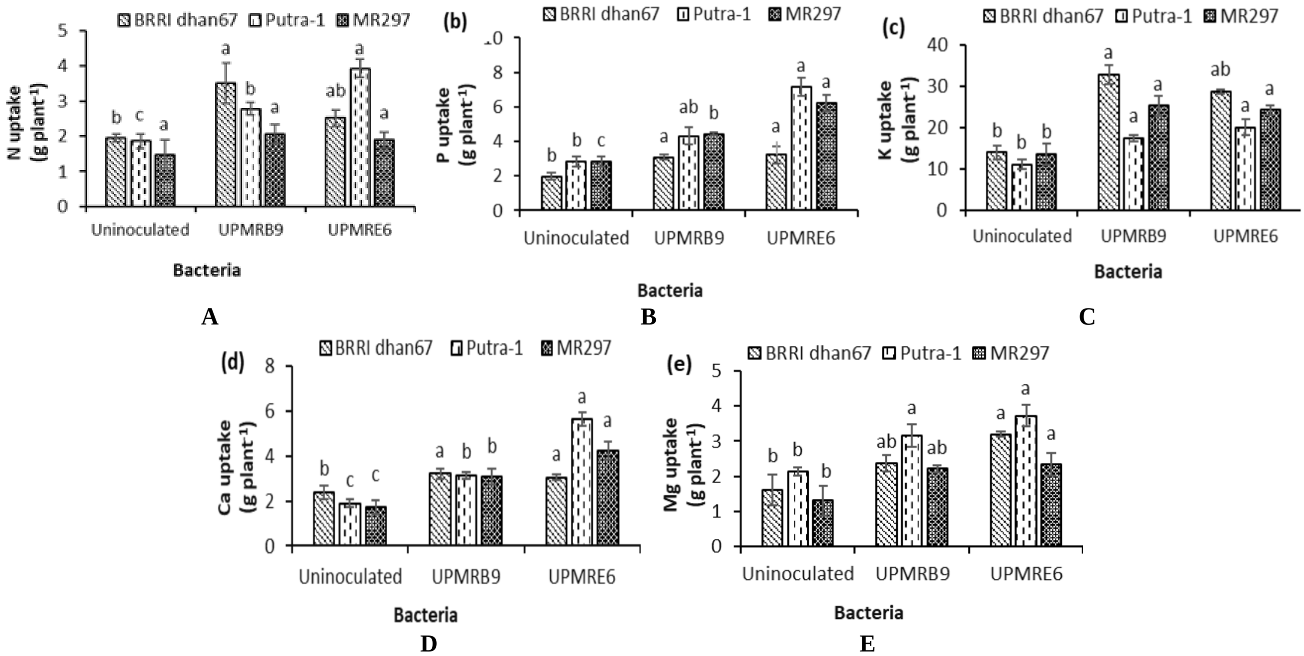

Figure 6. Effect of UPMRB9 and UPMRE6 inoculation on the uptake of: A. Nitrogen; B. Phosphorous; C. Potassium; D. Calcium and E. Magnesium in tested rice plants. Means having the same letter in each variety do not differ significantly using Tukey test at p>0.05

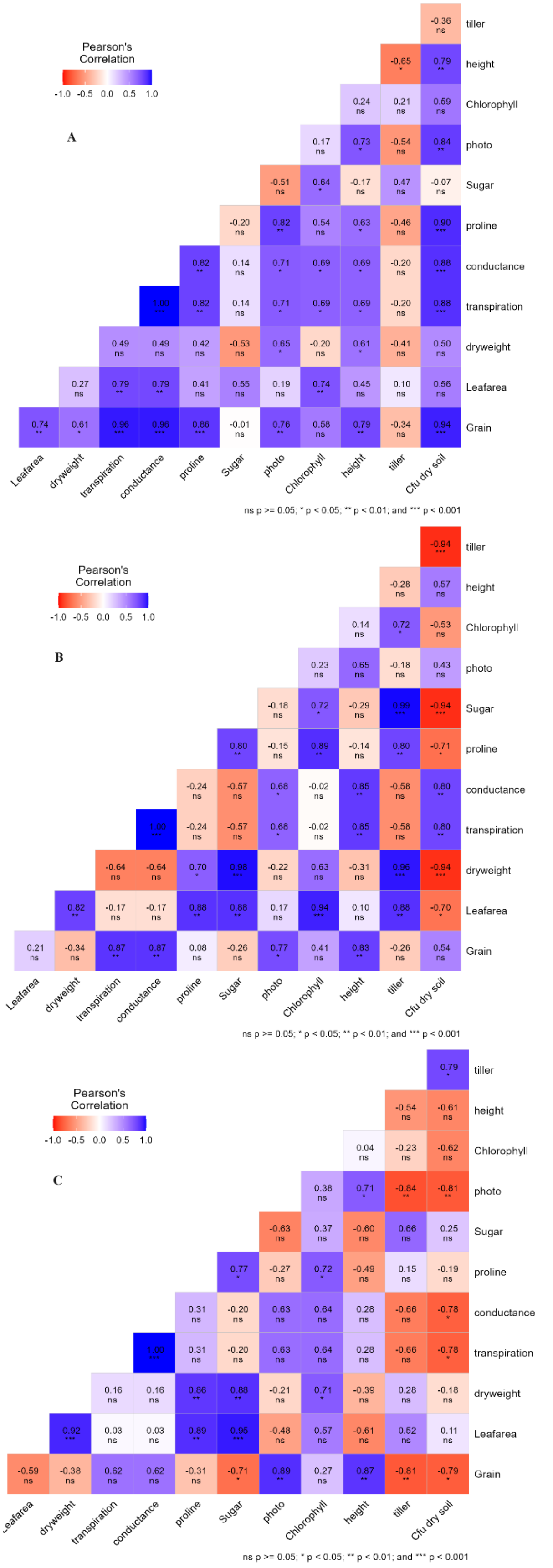

Figure 7. Correlation among yield and other traits. A. Association of UPMRE6 inoculation with three rice varieties; B. Association of UPMRB9 inoculation with three rice varieties; and C. Association of no inoculation with three rice varieties

The physiological properties of the three rice varieties varied significantly with bacterial inoculations. The MR297 variety inoculated with UPMRB9 exhibited the highest rate of photosynthesis. Putra-1 variety inoculated with UPMRB9 was recorded to enhance stomatal conductance. However, an accelerated rate of transpiration was observed in BRRI dhan67 without bacterial amendment. Previous investigations reported that by raising gas exchange parameters and chlorophyll fluorescence, inoculating plants with *B. subtilis* and *B. amyloliquefaciens* improved the photosynthetic performance of pepper plants. The bacteria were shown to facilitate an increased electron transfer rate in the thylakoid membranes, which in turn promoted photosynthetic capability (Samaniego-Gámez et al. 2016). The UPMRB9 strain could be beneficial for utilization in sustainable agricultural cultivation. A substantial amount of exopolysaccharide production by UPMRB9 aids in better colonization with Putra-1 roots.

Additionally, the higher photosynthetic ability in putra-1 results in the secretion of photosynthates (sugars) through the roots, fostering a stronger host-microbe relationship. Several researchers reported that the root colonization efficiency of PGPB is intimately linked to microbial competition and survival in the soil, as well as the regulation of gene expression and cell-cell communication via quorum sensing (Egamberdieva et al. 2017; Gamalero et al. 2022; Wang et al. 2022; Weemstra et al. 2022). Plant-growth-promoting rhizobacteria (PGPR) such as *Bacillus* spp. are widely dispersed in soil and produce strong endospores that are resistant to high temperatures and chemical concentrations (Bhat et al. 2023). *Bacillus* species improve biomass, yield, and seed emergence to promote plant growth. This is because of the synthesis of phytohormones like cytokinin, IAA, and gibberellin, and the increased uptake of available minerals, phosphorus, and nitrogen in the soil (Xie et al. 2014; Egamberdieva et al. 2017). Rice plants inoculated with PGPR exhibited profuse vegetative growth, with a wider root surface area, facilitating greater nutrient uptake, including Ca and Mg (Kaur et al. 2023). Through symbiotic relationships with plants, PGPR contributes to vigorous root growth by providing essential nutrients while utilizing root exudates as a food source (Vimal 2018; Weemstra et al. 2022).

In UPMRB9 inoculated conditions, positive and significant associations were observed among grain yield and parameters such as plant height, photosynthesis, conductance, and transpiration (Roy et al. 2024b). They also observed similar interactions between grain yields and other traits. This indicates that UPMRB9 inoculation positively influences these aspects, potentially leading to improved plant growth and productivity. The positive correlation with grain yield suggests that these factors contributed to increased yield under inoculated conditions. Interestingly, non-significant and negative associations indicated that inoculation might influence these parameters in a manner not conducive to higher grain yield. Multilocational field experiments addressing different climatic conditions could provide a better understanding of its activity under diverse natural conditions. However, several challenges could arise during its adoption under field conditions such as the shelf life of PGPR inoculum, selection of an appropriate carrier material, inoculum application techniques, climatic conditions and soil characteristics. Overcoming all the factors decides the successful establishment of a biofertilizer inoculum in a particular area.

In conclusion, the two potential indigenous strains, *Bacillus tequilensis* 10b and *Bacillus aryabhattai* B8W22 exhibited multiple plant growth-promoting properties. These strains significantly enhanced the growth of three rice varieties by improving morphological properties like plant height and leaf areas, physiological properties like photosynthesis, stomatal conductance and transpiration and biochemical properties like proline, total soluble sugar and total chlorophyll content which eventually triggered increased biomass and grain yield production of three rice varieties. Hence, to improve the existing rice production systems these potential isolates may be utilized as a source of biofertilizer. This glasshouse study serves as an initial basis for future large-scale field applications. However, substantial field experiments should be conducted before providing general recommendations.

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