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# Exploring the Phosphate Solubilising Rhizobacteria isolated from Wild *Musa* Rhizosphere and their Efficacy on Growth Promotion of *Phaseolus vulgaris*

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**ABSTRACT**

Plant growth-promoting rhizobacteria (PGPR) are recognized for enhancing plant growth, protecting against pathogens, and boosting productivity. The present study focused on isolating PGPR from the rhizosphere of wild *Musa*, screening for growth-promoting traits, and assessing their effects on the growth of *Phaseolus vulgaris* L. A total of 20 strains were isolated and evaluated for their capacity to solubilize phosphate, produce indole-3-acetic acid (IAA), synthesize siderophores, and their tolerance to salt and heavy metals. Among 20 isolates, four most effective isolates were selected and based on 16S rRNA sequencing these isolates were identified as: *Burkholderia cepacia* (RZ27), *Agrobacterium larrymoorei* (RZ23), *Pseudomonas taiwanensis* (RZ5), and *Pseudomonas orientalis (RZ3)*. *P. orientalis* exhibited the highest phosphate solubilization ability (222.17 µg/ml), followed closely by *B. cepacia* (222.80 µg/ml), *A. larrymoorei* (71.57 µg/ml), and *P. taiwanensis* (19.20 µg/ml). Isolate RZ27 demonstrated the greatest salt tolerance at 14%, followed by RZ5 and RZ23 (10% each) and RZ3 (6%). Notably, only isolate RZ23 produced IAA, while all isolates except RZ27 could produce siderophores. The highest siderophore production was recorded with RZ23 (33.34% siderophore production unit, SPU), followed by RZ3 (29.07 SPU) and RZ5 (27.20 SPU). *A. larrymoorei* and *P. orientalis* showed the highest chromium tolerance (1840 µg/ml), followed by *B. cepacia* (1810 µg/ml) and *P. taiwanensis* (1300 µg/ml). There was a noticeable enhancement in plant growth when *P. vulgaris* was inoculated with the PGPR strains. Among the four isolates, RZ3 significantly increased both shoot and root lengths and biomass compared to the control; meanwhile, isolate RZ23 improved shoot fresh weight. These findings suggest that these isolates have the potential to be used as bioinoculants to improve plant development.

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## 1 Introduction

Rhizobacteria found in the plant rhizosphere have the potential to improve plant growth. These beneficial microorganisms are plant growth-promoting rhizobacteria (PGPR) (Tatung and Deb 2024a; Deb and Tatung 2024; Megu et al. 2024a). PGPR interacts with plant roots and enhances growth through various mechanisms, including improved nutrient uptake (Qingwei et al. 2023), hormone production (Deb and Tatung 2024), siderophore production, and suppression of soil-borne diseases (Tatung and Deb 2023; 2024a, b; Qingwei et al. 2023; Deb and Tatung 2024; Megu et al. 2024a, b; Pongener et al. 2024).

Modern farming practices rely heavily on synthetic fertilizers and pesticides for higher yields. However, excessive chemical fertilizers can lead to soil degradation and environmental pollution (Aktar et al. 2021; Deb and Tatung 2024). Numerous studies have indicated that PGPR can mitigate the adverse effects of chemical fertilizers and serve as a viable alternative for sustainable agriculture (Tatung and Deb 2023; 2024a, b; Qingwei et al. 2023; Deb and Tatung 2024). For instance, Oo et al. (2020) reported that *Acromobacter insolitus* enhanced the seed germination of *Vigna radiata* and *Zea mays*, while *Pseudomonas plecoglossicida* increased the fresh weight of both crops. *Acromobacter insolitus* and *Enterobacter hormaechei* also improved maize and green gram root formation. Yamini et al. (2021) found that PGPR-derived phytohormones were more effective than crude hormones in promoting plant growth. Moreover, PGPR isolates such as *Staphylococcus* sp. and *Bacillus* sp. effectively ameliorated plant stress responses and enhanced the height of *Vallisneria natans* when subjected to sediment organic matter stress (Wang et al. 2021). In terms of stress adaptation, *Helianthus annuus*, and *Brassica juncea*, when grown under heavy metal contamination (Cd/Cu) and inoculated with PGPR consortia, showed improved growth characterized by enhanced shoot and root length, as well as increased fresh and dry weights (Tatung and Deb 2024a). In another study, Khanna et al. (2019) demonstrated that *Pseudomonas aeruginosa* and *Burkholderia gladioli* improved the seedling growth of *Lycopersicon lycopersicum* under Cd stress (0.4 mM). Additionally, *Bacillus aryabhattai* and *B. tequilensis*, both high salt-tolerant PGPR strains, boosted photosynthesis, transpiration, and stomatal conductance in rice plants, leading to increased yields (Shultana et al. 2020). Tatung and Deb (2023) noted that inoculating *Cicer arietinum* with *Kosakonia arachidis*, *Pseudomonas putida*, and *P. monteilii* increased growth compared to the control treatment. Lastly, Cupriavidus necator, either alone or in combination with *P. fluorescens*, improved shoot biomass, and enhanced phosphorus (P) and nitrogen (N) use efficiency in maize under water stress conditions (Pereira et al. 2020).

Cultivated *Musa* varieties are susceptible to various diseases, necessitating regular replacement of stock plants. In contrast, wild bananas tend to thrive year after year with minimal infection. This resilience may be attributed to plant growth-promoting rhizobacteria (PGPR) associated with the rhizosphere of wild *Musa*, which helps mitigate different pathogens affecting these plants (Tatung and Deb 2023, 2024a).

*Phaseolus vulgaris*, commonly known as common beans, is an important food legume globally, with an annual production value of $5.717 billion and a yield exceeding 12 million tons (FAO). However, the cultivation of common beans faces significant challenges, including high temperatures, drought, and various phytopathogens (Uebersax et al. 2023). Shockingly, approximately 143.88 million tons of chemical fertilizers are used worldwide to enhance crop production (Rana et al. 2011). Unfortunately, prolonged use of these fertilizers degrades soil quality and has adverse effects (Khurana and Kumar 2022). In light of these concerns, this study aimed to isolate PGPR from the rhizosphere of wild *Musa*, screen the isolates for beneficial traits, and conduct cross-inoculation of the selected isolates in *P. vulgaris* to investigate their potential for promoting plant growth.

## 2 Materials and Methods

### 2.1 Isolation of rhizobacteria

Rhizospheric soil was collected from the rhizosphere of *Musa balbisiana* at a depth of 15-20 cm from plants growing on the Nagaland University campus in Lumami, Nagaland, India. Care was taken to ensure the roots remained intact during the collection process. After gently shaking the soil from the roots, the collected soil was placed in sterile polythene bags to isolate rhizospheric bacteria further. A serial dilution technique was employed to isolate the rhizospheric bacteria. One gram of the soil sample was mixed with 10 ml of distilled water, and serial dilutions were carried out until a dilution of $10^{-5}$ was achieved. Forty milliliters of nutrient agar medium (composed of 20 g/L agar, 5 g/L sodium chloride, 10 g/L yeast extract, and 10 g/L peptide) was poured into Petri dishes. The medium was streaked with the diluted soil samples and incubated for three days at 28±2°C. Bacterial isolates were selected based on their physical characteristics and subcultured until pure cultures were obtained. A portion of these pure cultures was stored at -60°C in an 80% glycerol stock solution for future use.

### 2.2 Morphological studies and biochemical test of the bacterial isolates

Isolated bacterial strains were examined for their colony morphology characteristics, including elevation, shape, transparency, and colour. Twenty bacterial isolates from the mixed culture were subjected to a third-generation pure culture and used for biochemical tests. These tests included motility, Gram staining, methyl red testing, citrate

utilization, starch hydrolysis, catalase production, and sugar fermentation ability, as described by Tatung and Deb (2023).

### 2.3 Evaluation for plant growth promoting characteristics of isolates

To evaluate the isolated rhizobacteria as potential plant growth-promoting rhizobacteria (PGPR), they were subjected to tests for heavy metal (chromium) tolerance, salinity tolerance, IAA production, siderophore production, and phosphate solubilization capability.

#### 2.3.1 Qualitative estimation of phosphate solubilization

The phosphate (NBRIP) growth medium developed by the National Botanical Research Institute was utilized to evaluate the phosphate solubilization capability of certain isolates. The culture medium consisted of the following components: 10 g/L glucose, 5 g/L $Ca_3(PO_4)_2$, 5 g/L $MgCl_2.6H_2O$, 0.25 g/L $MgSO_4.7H_2O$, 0.2 g/L KCl, 0.2 g/L $(NH_4)_2SO_4$, and 15 g/L water (You et al. 2020). The isolates were cultured on NBRIP agar plates for 7 days at 28 ± 2ºC. Phosphate-solubilizing isolates were identified by a distinct halo zone surrounding their colonies.

#### 2.3.2 Quantitative assay of phosphate solubilization

Quantitative analysis of phosphate solubilization was conducted in a liquid NBRIP medium, following the method outlined by Pande et al. (2017). Phosphate solubilization was measured using 10 ml of NBRIP broth, which had the following composition (in g/L): 10 g of glucose, 5 g of $Ca_3(PO_4)_2$, 5 g of $MgCl_2.6H_2O$, 0.25 g of $MgSO_4.7H_2O$, 0.2 g of KCl, 0.1 g of $(NH_4)_2SO_4$, with the pH adjusted to 7.0. A control was prepared using NBRIP broth alone. Freshly cultured bacterial colonies were incubated in the NBRIP broth for 12 days at 28 ± 2ºC to test the strains.

After incubation, 1 ml of supernatant was collected on the 2$^{nd}$, 4$^{th}$, 6$^{th}$, 8$^{th}$, 10$^{th}$, and 12$^{th}$ days. The suspension cultures were centrifuged at 10000 rpm for 5 minutes, and the supernatants were collected and filtered for quantification. Approximately 600 µL of the filtered supernatant was mixed with 1500 µL of Barton's reagent, and the volume was adjusted to 5 ml with double-distilled water. This mixture was allowed to rest for 10 minutes. After the resting period, the intensity of the yellow colour was measured using a spectrophotometer (Thermo Scientific Multiskan Go) at a wavelength of 430 nm. The amount of phosphate solubilized was determined from a standard curve. The experiments were conducted in triplicate, and the results were expressed as mean values.

#### 2.3.3 Qualitative analysis of siderophore production

For qualitative analysis of siderophore production, isolates were cultured on CAS agar medium for seven days (Tatung and Deb 2023). The presence of an orange ring surrounding the bacterial colonies indicated siderophore production.

#### 2.3.4 Quantitative analysis of siderophore production

Each isolate was cultured in CAS nutrient broth for 10 days at 28±2ºC. After the culture period, 2 µL samples from each isolate were collected every two days and centrifuged at 1000 rpm for 10 minutes. The supernatant was then collected, and its absorbance was measured at 630 nm using a microplate reader. The production of siderophores by the strains was calculated using the formula provided by Payne (1993):

$$\text{Percent Siderophore Unit (PSU)} = \frac{(A_r - A_s)\ X100}{A_r}$$

Where $A_r$ - Reference Absorbance (Uninoculated broth +CAS reagent), $A_s$ - Sample Absorbance (Sample's Cell-free supernatant +CAS solution).

#### 2.3.5 IAA production

For the qualitative assessment of indole-3-acetic acid (IAA) production, 10 ml of nutrient broth supplemented with 0.1% w/v tryptophan was inoculated with freshly grown bacterial cultures and incubated at a temperature of 28±2ºC for 7 days. A control was set up using 10 ml of nutrient broth with 0.1% tryptophan but without bacterial inoculation. On the 7$^{th}$ day, 1 ml of the culture was taken and centrifuged for 5 minutes at 10000 rpm. The supernatant was then transferred to a vial containing 2 ml of Salkowski reagent. After incubating for 25 minutes, cultures that produced a pink colour were identified as positive for IAA production, while those that did not show any colour change were considered negative (Tatung and Deb 2023).

#### 2.3.6 Salinity tolerance

The salinity tolerance level of the isolates was tested following Sharma et al. (2021a). Bacterial isolates were cultured on a nutrient agar medium fortified with different concentrations of sodium chloride (NaCl) (2-14%) with an increment of 2% and cultured for 72 h at 28±2ºC. Isolates continued to grow on the NaCl-enriched medium, indicating their tolerance level.

#### 2.3.7 Heavy metal tolerance (Chromium)

Bacterial isolates were evaluated for heavy metal tolerance using the minimum inhibitory concentration (MIC) method (Yadav et al. 2022). Nutrient agar plates were supplemented with various chromium concentrations, ranging from 30 to 2000 µg/ml in increments of 30 µg. The plates were streaked with the bacterial isolates and incubated at 28±2°C for 72 hours. A negative control plate without chromium was also inoculated and incubated for comparative purposes.

### 2.4 Molecular characterization of the isolates

Colony PCR targeted the 16S rRNA gene for partial sequencing to identify bacterial isolates. Freshly streaked bacterial cultures were grown on nutrient agar media for 24 hours. Colonies were picked using disinfected toothpicks and then suspended in 60 µL of Triton X-100 buffer in PCR tubes. This suspension was boiled for 10 to 15 minutes, followed by freezing for 2 to 3 minutes. After freezing, the samples were centrifuged for 3 to 4 minutes at 10000 rpm. PCR amplification of the target sequence was conducted using specific primers: the 1492R reverse primer and the 18F forward primer, as described by Tatung and Deb (2024a). The PCR mixture included 0.6 µL of dNTPs, 3 µL of buffer, 21.7 µL of sterile deionized water, 3 µL of the template, and 1 µL of Taq DNA polymerase. The PCR reaction was carried out using a Bio-Rad thermal cycler, starting with an initial denaturation at 95ºC, followed by 30 cycles consisting of denaturation at 94ºC for 50 seconds, annealing at 55ºC for 90 seconds, and extension at 72ºC for 1 minute, concluding with a final extension at 72ºC for 3 minutes. The PCR products were analyzed using a 1% (w/v) agarose gel and subsequently sequenced. The resulting gene sequences were compared with those in the GenBank database using NCBI BLAST. The sequences were then submitted to the NCBI GenBank database for accession numbers. A phylogenetic tree was created using MEGA11 software.

### 2.5 Pot experiment

The growth-promoting abilities of four bacterial isolates were evaluated using *Phaseolus vulgaris* as a model plant. The isolates included strain RZ3 (*Pseudomonas orientalis*), strain RZ5 (*Pseudomonas taiwanensis*), strain RZ23 (*Aureobasidium larrymoorei*), and strain RZ27 (*Burkholderia cepacia*). A fully randomized design was implemented, featuring a control group and four treatment groups: C (control with no PGPR), RZ3, RZ5, RZ23, and RZ27. The potting mixture was prepared using a 1:1 ratio of soil and sand. This soil-sand mixture was then disinfected by autoclaving for 30 minutes at 121 psi before being placed in plastic pots. In each pot, 10 sterilized seeds were inoculated with different rhizobacterial isolates and allowed to germinate. After germination, the seedlings were thinned to three plants per pot. The control pots contained non-inoculated seeds. The plants were watered regularly with sterilized tap water. After 30 days, the effects of the rhizobacteria on seedling growth were assessed. The plants were uprooted, and growth parameters, including root and shoot lengths and the fresh and dry weights of both shoots and roots, were compared.

### 2.6 Statistical analysis

Statistical analysis of the data was conducted using IBM SPSS Statistics software. The results are presented as the mean of three replicates ± standard error of the mean (SEM). The data were further analyzed with a one-way ANOVA. For significant F values, post hoc comparisons were carried out using the Least Significant Difference (LSD) test at a significance level of $P \leq 0.05$. Graphs were created using Microsoft Excel software.

## 3 Results

### 3.1 Isolation of the PGPR

From the mixed culture plates, 20 bacterial isolates were selected based on the morphological characteristics of bacterial colonies for raising pure cultures (Table 1). Third-generation pure cultures of the rhizobacterial isolates were considered for biochemical analysis (Table 2). Growth-promoting traits such as phosphate solubilization, IAA and siderophore production, chromium, and salt tolerance were tested for all isolated isolates. Subsequently, four best-performing bacterial isolates (R27, RZ23, RZ5, and RZ3) were chosen for inoculation onto *P. vulgaris* to evaluate their effects on various growth parameters.

### 3.2 Morphological and biochemical analysis of the bacterial isolates

Colony morphology characteristics, such as size, shape, colour, and growth pattern, were evaluated after 24 hours of growth on nutrient agar plates. There was significant variation in the colour and transparency of the bacterial colonies. All isolates, except for RZ23, which had a yellow colony, appeared off-white and opaque. Isolate RZ27 formed a raised colony, while the other three isolates produced flat colonies. The margins of the colonies also varied among the isolates; RZ27 had a regular margin, RZ23 had an entire margin, RZ5 had an undulate margin, and RZ3 exhibited a serrated margin. All isolates had round colonies except for RZ3, which had an irregular shape.

Table 1 Colony morphology of the bacterial isolates

| Bacterial Isolates | Margin | Elevation | Shape | Colour | Transparency | Identification | Gen Bank Accession No. |
|---|---|---|---|---|---|---|---|
| RZ27 | Regular | Raised | Round | Off-white | Opaque | *B. cepacian* | OL662932 |
| RZ23 | Entire | Flat | Round | Yellowish | Opaque | *A. larrymoorei* | OL662933 |
| RZ5 | Undulate | Flat | Round | Off-white | Opaque | *P. taiwanensis* | OL662931 |
| RZ3 | Serrated | Flat | Irregular | Off-white | Opaque | *P. orientalis* | OL662936 |

Table 2 Biochemical analysis of the isolated bacterial isolates

| Bacterial isolates | Gram staining | Motility | Starch hydrolysis | Catalase test | Methyl red | Citrate utilization |
|---|---|---|---|---|---|---|
| RZ27 | - | + | + | + | - | - |
| RZ23 | - | + | - | + | - | - |
| RZ5 | - | + | - | + | - | + |
| RZ3 | - | + | + | + | - | + |

'+' indicates positive, '- 'indicates negative

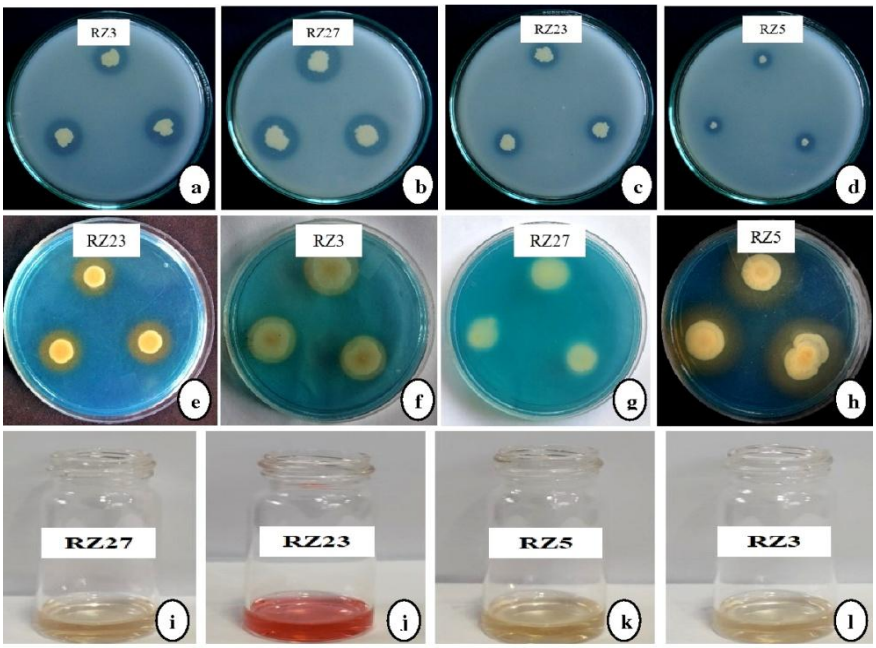


Figure 1 (a-d) Phosphate solubilization by the bacterial isolates a. RZ3, b. RZ27, c. RZ23, and d. RZ5 on NBRIP agar media indicated the development of a clear halo zone around the bacterial colony. e-h: Siderophore production test by the bacterial isolates on CAS-agar media e. RZ23, f. RZ3, g. RZ27, and h.RZ5. i-l: IAA production test by the bacterial isolates in Nutrient broth supplemented with 0.1% of L-tryptophan by the bacterial isolates i.RZ27, j.RZ23, k.RZ5, and l.RZ3.

Biochemical analysis indicated that all four isolates were gram-negative. They showed negative results in the methyl red test but tested positive in the catalase test and were found to be motile. Isolates RZ27 and RZ3 tested positive for starch hydrolysis, while RZ23 and RZ5 tested negative. Furthermore, isolates RZ27 and RZ23 were negative for citrate utilization, whereas RZ5 and RZ3 were positive (Tables 1 and 2).

### 3.3 Plant growth promoting screening

#### 3.3.1 Phosphate solubilization

After incubating for 7 days on NBRIP agar plates containing tricalcium phosphate [$Ca_3(PO_4)_2$] as the sole phosphate source, 12 bacterial isolates demonstrated phosphate solubilizing activity. Four isolates with the highest phosphate solubilizing ability were taken for further analysis (Figure 1 a-d). Quantitative analysis revealed that isolate RZ3 exhibited the highest phosphate solubilization (337.11±0.58µg/ml), followed by RZ27 (222.80±0.30µg/ml), RZ23 (71.57±0.56µg/ml), and RZ5 (19.20±0.33µg/ml). It was observed that with the increase of phosphate in the medium, the pH decreased, and among the tested isolates, culture with isolate RZ3 was found to have the lowest pH (4.23±0.03), followed by RZ27 (4.45±0.03), RZ23 (5.53±0.02), and RZ5 (6.23±0.01) (Tables 3 and 4).

#### 3.3.2 Siderophore production

When tested for siderophore production on CAS agar plates, all isolates except RZ27 registered positive results (Figure 1 e-h). The development of an orange halo zone surrounding the colonies indicated siderophore production ability and served as a marker for positive isolates. Quantitative analysis revealed that RZ23 had the maximum production of siderophore (33.34±0.03µg/ml) among the three positive isolates, followed by RZ3 (29.07±0.09µg/ml) and RZ5 (27.20±0.02µg/ml) on the 10th day of incubation.

Table 3 Growth-promoting traits of the bacterial isolates

| Bacterial Isolates | Identification | IAA Production | Phosphate Solubilization | Siderophore Production | NaCl Tolerance (%) | Chromium Tolerance (µg/ml) |
|---|---|---|---|---|---|---|
| RZ27 | *B. cepacian* | - | + | - | 14 | 1810 |
| RZ23 | *A. larrymoorei* | + | + | + | 10 | 1840 |
| RZ5 | *P. taiwanensis* | - | + | + | 10 | 1300 |
| RZ3 | *P. orientalis* | - | + | + | 6 | 1840 |

Note: '+': Indicates positive, '- ': Indicates negative.

Table 4 Quantification of phosphate solubilization characteristics of the selected bacterial isolates under culture condition

| Bacterial Isolates | Concentration of $PO_4$ (µg/ml) | | | | | | pH of the medium | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2nd day | 4th day | 6th day | 8th day | 10th day | 12th day | 2nd day | 4th day | 6th day | 8th day | 10th day | 12th day |
| RZ27 | 43.18± 0.25 | 88.41± 0.09 | 109.79±0.77 | 165.27± 1.07 | 222.80± 0.32 | 108.09± 0.13 | 4.95±0.03 | 4.71± 0.01 | 4.54± 0.02 | 4.47± 0.04 | 4.45± 0.03 | 4.24± 0.01 |
| RZ5 | 18.83± 0.09 | 19.20± 0.33 | 18.46± 0.17 | 17.66± 0.34 | 15.24± 0.04 | 14.32± 0.04 | 6.32± 0.01 | 6.23± 0.01 | 6.41± 0.03 | 6.49± 0.01 | 6.51± 0.04 | 6.51± 0.02 |
| RZ23 | 19.28± 0.31 | 29.24± 0.43 | 46.86± 0.28 | 57.47± 0.41 | 64.85± 0.70 | 71.57± 0.56 | 5.93± 0.02 | 5.85± 0.01 | 5.34± 0.01 | 5.31± 0.02 | 5.25± 0.01 | 4.23± 0.03 |
| RZ3 | 78.54± 0.60 | 129.09± 0.43 | 187.55± 1.66 | 199.92± 0.95 | 337.11± 0.58 | 324.88± 2.63 | 6.16± 0.02 | 5.93± 0.01 | 5.76± 0.01 | 5.64± 0.03 | 5.53± 0.02 | 5.47± 0.02 |

* Data was expressed as mean values of the three replicates with standard error.

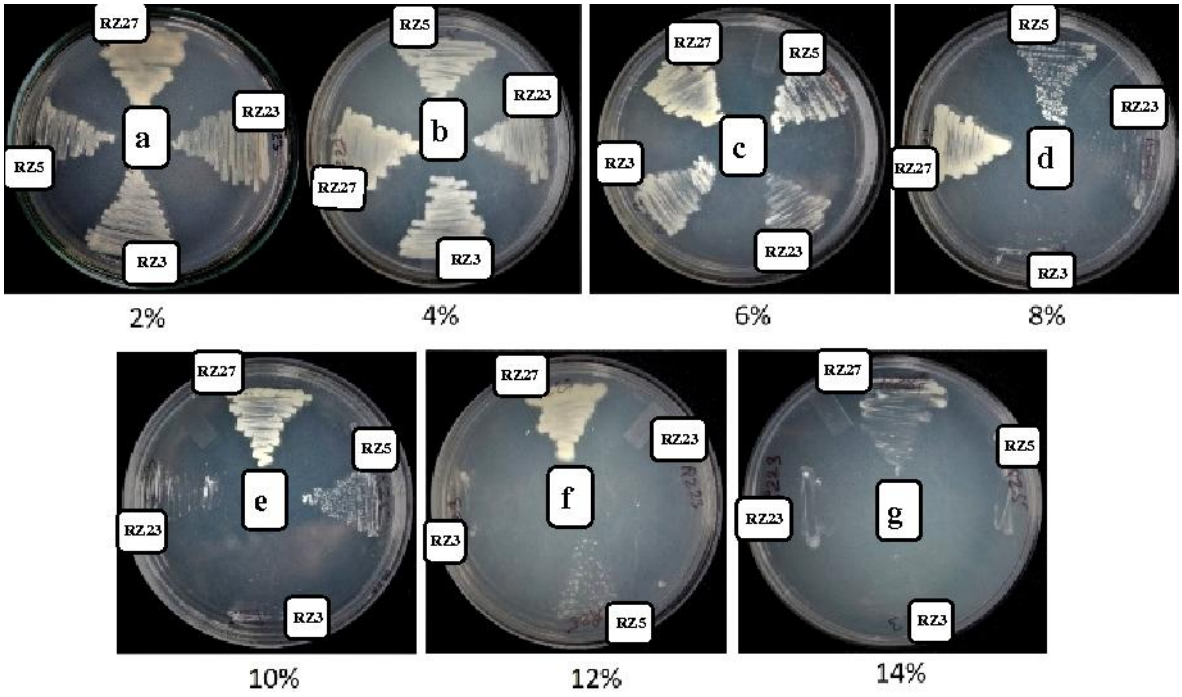


Figure 2 Salinity tolerance test of the bacterial isolates RZ27, RZ23, RZ5, and RZ3 on nutrient agar medium supplemented with different concentrations of NaCl (a. 2%, b. 4%, c. 6%, d. 8%, e. 10%, f. 12% and g. 14%, w/v).

**3.3.3 Production of indole-3acetic acid**

Bacterial isolates underwent qualitative testing for IAA production, revealing that isolate RZ23 only produced IAA, and the remaining three isolates did not register any production (Figure 1 i-l and Table 3).

**3.3.4 Bacterial growth under different concentrations of NaCl**

When tested for salt tolerance level, all four strains were tolerant of NaCl at various levels. Amongst the four isolates, isolate RZ27 exhibited the highest tolerance, thriving up to 14% NaCl, followed by isolates RZ23 and RZ5 (each up to 10%) and RZ3 (6%) (Figure 2 and Table 3).

**3.3.5 Heavy metal tolerance (chromium)**

Various studies have extensively used plant growth-promoting rhizobacteria in the phytoremediation of heavy metal-contaminated soils. Heavy metals like chromium (Cr) are essential micronutrients for microbes, plants, and animals at lower concentrations. However, these metals become major toxins for all life forms at higher levels. In the present study, the chromium tolerance ability of the four selected isolates was tested, and the result revealed that different isolates had different tolerance levels of chromium. Amongst the four isolates, isolates RZ23 and RZ23 registered tolerance up to 1840µg/ml, followed by isolate RZ27 (1810µg/ml) and RZ5 (1300µg/ml). Heavy metal concentrations on nutrient agar plates were gradually increased until the strains failed to grow. Cultures that grew at the highest concentration were subsequently transferred to plates with even higher concentrations. The MIC was determined when the isolates failed to grow Table 3).

**3.4 Molecular characterization of bacterial isolates**

From the mixed culture plates, 20 rhizobacterial isolates were selected for pure cultures. Based on biochemical analysis, the best performing four isolates were subjected to molecular characterization based on *16S rRNA* sequence. Based on the targeted sequence, the isolates were confirmed RZ23 as *Agrobacterium larrymoorei* (GenBank accession No. OL662933), RZ5 as *Pseudomonas taiwanensis* (OL662931), RZ3 as *Pseudomonas orientalis* (OL662936), and RZ27 as *Burkholderia cepacian* (OL662932) (Table 1). Using Mega11 Software, a phylogenetic tree was built based on BLAST analysis to identify these bacterial species' closest relatives and homology (Figure 3).

**3.5 Effect of bacterial inoculation on the growth of *Phaseolus vulgaris* L.**

After 30 days of planting, the seedlings were uprooted, and their growth parameters were assessed. It was observed that all four rhizobacterial strains significantly enhanced various growth parameters of *Phaseolus vulgaris* seedlings in comparison to the control group (Figures 4 and 5). Each bacterial isolate positively

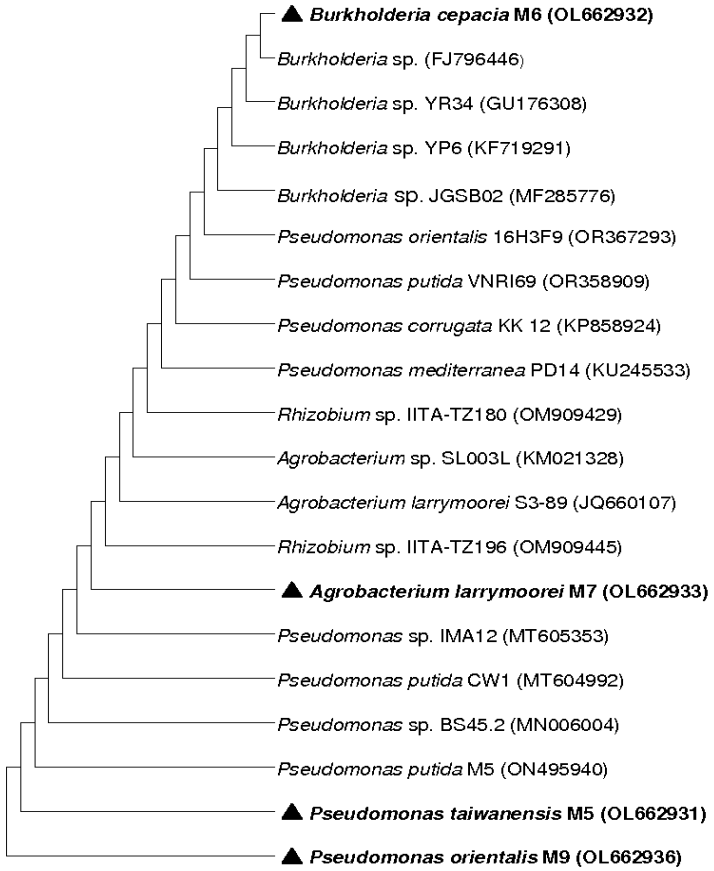


Figure 3 Neighbor-Joining method was used to infer the evolutionary history. The evolutionary distances were calculated using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site.

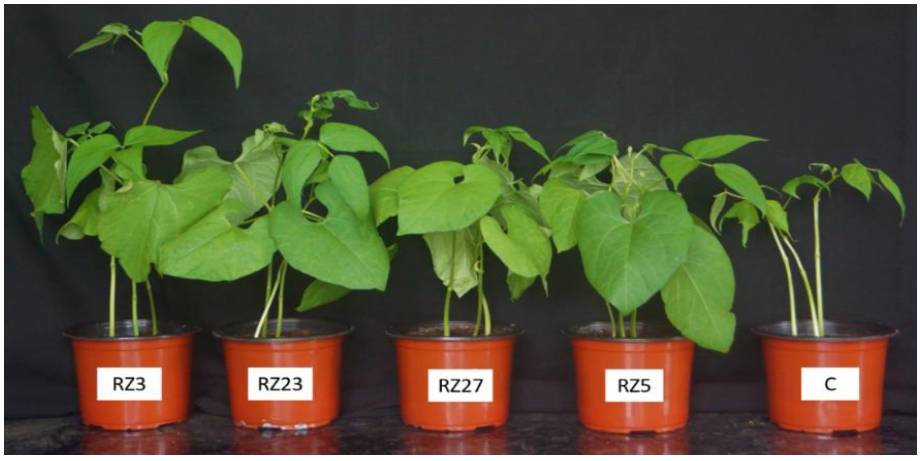


Figure 4 Plant growth promotion assay of common bean plant (*Phaseolus vulgaris* L.) by bacterial isolates (after 30 days post inoculation with strains a. RZ3, b. RZ23, c. RZ27, d. RZ5 and e. CONTROL (C).

influenced one or more growth parameters of the experimental plants. Among the tested isolates, isolate RZ3 emerged as the best performer under the experimental conditions for all studied parameters, including shoot length (65.33 cm), root length (40.67 cm), shoot fresh weight (9.70 g), root fresh weight (6.48 g), shoot dry weight (1.93 g), and root dry weight (0.78 g). These values were considerably higher than those of the control treatment, which recorded a shoot length of 14.00 cm, root length of 12.67 cm, shoot fresh weight of 1.46 g, root fresh weight of 0.85 g, shoot dry weight of 0.36 g, and root dry weight of 0.37 g.

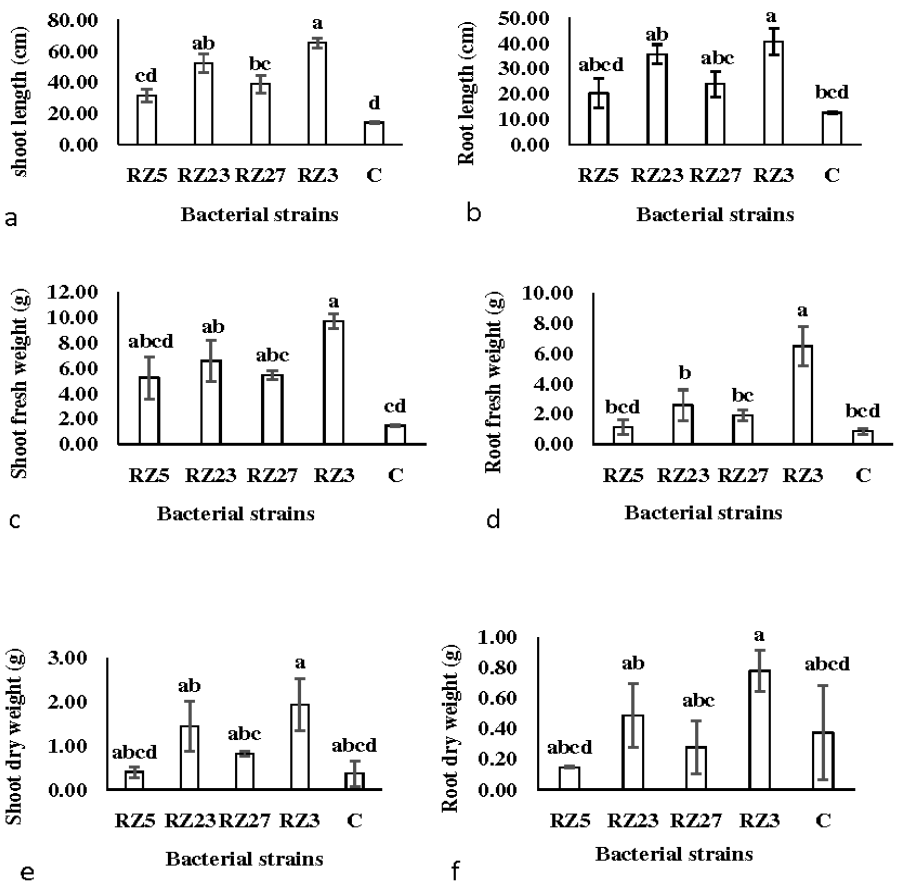


Figure 5 Effect of different bacterial isolates on shoot and root growth of *P. vulgaris* after 30 days of growth. a. Shoot length, b. Root length, c. Shoot fresh weight, d. Root fresh weight, e. Shoot dry weight and f. Root dry weight. Note: The PGPR isolates were RZ5, RZ23, RZ27, RZ3 and C (Control treatment). Mean averages (n=3). Different letters on bars indicate statistical differences between treatments according to the LSD Test ($p \leq 0.05$).

Isolate RZ23 significantly improved shoot length (52.33 cm) and shoot fresh weight (6.57 g). Increases in root length (35.67 cm), root fresh weight (2.59 g), shoot dry weight (1.44 g), and root dry weight (0.49 g) were also observed with RZ23, although these increases were not statistically significant ($p \geq 0.05$). Isolate RZ27 showed a significant increase only in shoot length (39.00 cm), with no significant changes in the other parameters. Similarly, isolate RZ5 did not exhibit any significant improvements in any of the growth parameters.

**4 Discussion**

In modern agriculture, a significant concern is the reliance on chemical fertilizers, which emphasizes the need for sustainable practices that reduce their use. This underscores the importance of exploring alternative methods to maintain agroecosystems while protecting the environment (Tatung and Deb 2021; 2023; 2024a, b; Deb and Tatung 2024; Yaghoubi et al. 2024). One promising solution lies in biofertilizers, biopesticides, and bioinsecticides, which are eco-friendly, renewable, and do not lead to resistance in target organisms.

In the present study, plant growth-promoting rhizobacteria (PGPR) strains were isolated from the rhizosphere of *Musa balbisiana* growing in the forests of Nagaland. Their plant growth-promoting traits characterized these strains. Four promising isolates were identified among the experimental parameters evaluated: *A. larrymoorei*, *B. cepacian*, *P. taiwanensis*, and *P. orientalis*. These isolates were then tested on a model crop plant to assess their effect on plant growth.

After nitrogen, phosphate is the second most important macronutrient for plant growth and development. Although soils often contain abundant phosphate, only a small fraction (approximately 0.1%) is soluble and available for plant uptake (Hadjouti et al. 2022; Tatung and Deb 2023). Phosphate-solubilizing bacteria (PSB) are vital in converting insoluble inorganic phosphorus into soluble orthophosphates (Sanchez-Gonzalez et al. 2022).

In this study, all four selected bacterial strains demonstrated the ability to solubilize phosphate. Among them, *P. orientalis* exhibited the highest phosphate solubilization capability (337.11 µg/ml) after 10 days of culture, followed by *B. cepacian* (222.80 µg/ml after 10 days), *A. larrymoorei* (71.57 µg/ml after 12 days), and *P. taiwanensis* (19.20 µg/ml after 4 days). The quantification of available soluble phosphate in the culture medium was conducted using a standard curve prepared with various concentrations of $KH_2PO_4$ (0-600 µg/ml). The results showed that as the quantity of soluble phosphate in the medium increased, the pH decreased proportionately due to the accumulation of organic acids. This is a key mechanism for quantifying microbial phosphate solubilization (Sanchez-Gonzalez et al. 2022).

According to Chen et al. (2006), the solubilization of insoluble tricalcium phosphate largely relies on PSB and the reduction of pH in the NBRIP medium. In our findings, the lowest pH was observed with isolate RZ23 (*A. larrymoorei*) at 4.23 on the 12$^{th}$ day, closely followed by RZ27 (*B. cepacian*) at 4.24 on the 12$^{th}$ day, RZ3 (*P. orientalis*) at 5.47 on the 12$^{th}$ day, and RZ5 (*P. taiwanensis*) at 6.23 on the 4$^{th}$ day. Pande et al. (2017) reported that increased medium acidification accelerates mineral solubilization.

Phytohormones are essential chemical messengers that, although present in low concentrations, play a significant role in the growth and development of plants. They influence seed germination, flowering time, gender determination, leaf senescence, and fruit development (Tatung and Deb 2021). Among the four isolates tested, only *A. larrymoorei* could produce indole-3-acetic acid (IAA) in vitro, while the other three isolates did not show any colour change in the IAA production test.

Isolate *A. larrymoorei*, a relatively new species within the genus *Agrobacterium*, was first reported to induce tumours in the branches of *Ficus benjamina* (Bouzar and Jones 2001). It can be distinguished biochemically and genetically from other *Agrobacterium* species (Molinaro et al. 2003). An alternative name, *Rhizobium larrymoorei*, was proposed based on Rule 34a of the International Code of Nomenclature of Bacteria (Prokaryotes) (Young 2004). Despite being known for over 20 years, there has been limited research on *A. larrymoorei*, particularly regarding its potential for promoting plant growth. This study has established that this species can be considered a Plant Growth-Promoting Rhizobacterium (PGPR).

Although the Earth's crust contains a significant amount of iron, its bioavailability in aerobic environments is limited due to the low solubility of iron (III) (Hider and Kong 2010; Deb and Tatung 2024). Siderophores are small extracellular organic compounds with low molecular weight, secreted by microorganisms and some grass species under iron-deficient conditions. These compounds facilitate iron acquisition from the environment by solubilizing and transporting it (Ferreira et al. 2019).

In the current investigation, among the four isolates studied, only *B. cepacia* did not produce siderophores, while the other three isolates demonstrated siderophore production. This indicates that the positive isolates are effective in plant nutrient acquisition and have potential as biocontrol agents (Tatung and Deb 2021; 2023). The isolates that produced siderophores formed orange halo zones around their colonies on CAS-agar plates. This colour change occurs because the siderophores remove iron from the Fe-CAS complex, which is initially blue (Alexander and Zubber 1991).

Quantitative analysis revealed that *P. orientalis* showed the highest siderophore production on the 4$^{th}$ day, with a concentration of 29.07 µg/ml. This was followed by lower productions of 6.17 µg/ml on the 2$^{nd}$ day and 13.30 µg/ml on the 6$^{th}$ day, and no siderophore was detected on the 8$^{th}$ and 10$^{th}$ days. For *A. larrymoorei*, peak siderophore production also occurred on the 4$^{th}$ day at 33.34 µg/ml, with levels of 24.44 µg/ml on the 2$^{nd}$ day, 31.39 µg/ml on the 6$^{th}$ day, 25.75 µg/ml on the 8$^{th}$ day, and the lowest concentration of 18.00 µg/ml on the 10$^{th}$ day. *Pseudomonas taiwanensis* exhibited its highest siderophore production on the 4$^{th}$ day at 27.20 µg/ml, with lower levels of 14.70 µg/ml on the 2$^{nd}$ day, 20.96 µg/ml on the 6$^{th}$ day, and 17.08 µg/ml on the 8$^{th}$ day, and no siderophores detected on the 10$^{th}$ day.

Salinity stress significantly affects plant growth by impairing nitrogen metabolism, carbon acclimatization, and crop yield. It leads to osmotic imbalance and ionic toxicity, which hinders nutrient absorption (Li et al. 2020). Additionally, salinity stress generates reactive oxygen species (ROS), such as hydrogen peroxide ($H_2O_2$), superoxide ions, and singlet oxygen. These reactive species reduce the activity of plant defence enzymes, disrupt sodium balance, impair iron uptake, and alter the levels of phenols and trace elements (Sharma et al. 2021a).

In this investigation, *Burkholderia cepacia* exhibited the highest salt tolerance at 14% NaCl, followed by *Azospirillum larrymoorei* and *Pseudomonas taiwanensis*, which tolerated up to 10% NaCl. In contrast, *Pseudomonas orientalis* could withstand only up to 6% NaCl. The ability of rhizobacteria to adapt to salt stress is essential for their survival and growth in saline environments (Bakhshandeh et al. 2014; Tatung and Deb 2023). During salinity stress, plant growth-promoting rhizobacteria (PGPR) either activate or modulate plant response systems or produce anti-stress compounds (Giannelli et al. 2023). For instance, *Burkholderia* species have been reported to enhance several biochemical and morphological characteristics of rice seedlings under salinity stress compared to control treatments (Sarkar et al. 2018). Utilizing salt-tolerant PGPR can mitigate the harmful effects of salt stress on plants,

helping to improve physiological functions such as growth, yield, and disease resistance (Sharma et al. 2021a).

Chromium is one of the most toxic heavy metals, commonly found in nature and extensively used in industrial processes (Prasad et al. 2021). The Environmental Protection Agency sets the soil concentration limit for chromium between 95 and 1180 ppm (Mazhar et al. 2020). Chromium adversely affects plant growth by disrupting essential metabolic processes, primarily by producing reactive oxygen species (ROS), which induce oxidative stress in plants (Sharma et al. 2021b).

In the present study, rhizobacterial isolates showed varying levels of chromium tolerance: *A. larrymoorei* and *P. orientalis* tolerated up to 1840 µg/ml, followed by *B. cepacia* (1810 µg/ml) and *P. taiwanensis* (1300 µg/ml). According to Janaki et al. (2024), *B. cepacia* has demonstrated resistance to heavy metals like cadmium and lead, improving seed germination, plant height, plant biomass, and various enzyme activities, including peroxidase (PO), polyphenol oxidase (PPO), β-1,3-glucanase, and phenols. Furthermore, Kang et al. (2017) showed that inoculating *Brassica rapa* with *B. cepacia* enhanced plant growth, reduced zinc uptake, altered amino acid regulation, and increased flavonoid and phenolic levels. This inoculation significantly decreased levels of superoxide dismutase, endogenous abscisic acid, and salicylic acid, highlighting its bioremediation potential. These findings underline the plant growth-promoting and bioremediation capabilities of *B. cepacia*.

Molecular characterization based on the 16S rRNA sequence confirmed the identity of the four investigated isolates: *B. cepacia* (RZ27), *A. larrymoorei* (RZ3), *P. taiwanensis* (RZ5), and *P. orientalis* (RZ3), with homologies of 98.74%, 98.69%, 99.78%, and 100.00%, respectively, as determined using MEGA X software for phylogenetic analysis.

In this study, the selected PGPR strains were tested for their ability to promote the growth of *P. vulgaris*, a well-known bean crop plant exhibiting significant growth enhancements. Among the five treatments, *P. orientalis* resulted in the greatest improvements in both shoot and root length and in shoot and root fresh weight. This was followed by *A. larrymoorei*, *B. cepacia*, and *P. taiwanensis*, all of which performed better than the control treatment. These findings align with those reported by Mishra et al. (2023), who noted that *P. taiwanensis* improved wheatgrass's antioxidant and nutritional properties (*Triticum aestivum*) when used with reduced mineral fertilizers in saline soil. Additionally, *A. larrymoorei* exhibited significant plant growth-promoting traits, including phosphate solubilization, siderophore, and IAA production, indicating its potential to enhance plant growth alongside the other three isolates.

The growth-promoting traits of *B. cepacia* were also investigated by Wang et al. (2021), who observed its positive effects on maize growth. You et al. (2020) reported that *B. cepacia* could effectively solubilize inorganic tricalcium phosphate under greenhouse conditions and contribute to crop yield. Furthermore, *B. cepacia* has been shown to promote rice growth by increasing grain yield and biomass under nitrogen-limited conditions (Li et al. 2022). Rhizobacteria such as *P. orientalis* and *Chaetomium cupreum*, when inoculated on *Eucalyptus globulus*, enhanced plant growth and alleviated copper toxicity (Ortiz et al. 2019). Collectively, these findings highlight the potential of these PGPR strains, isolated from the *Musa* rhizosphere, as promising bioinoculants for sustainable agriculture.

**Conclusions**

The findings of this study highlight that the wild *Musa* rhizosphere harbors a diverse range of PGPR with the ability to thrive under adverse conditions, thus boosting the growth and productivity of plants. The investigated isolates *P. orientalis* (OL662936), *A. larrymoorei* (OL662933), *B. cepacian* (OL662932), and *P. taiwanensis* (OL662931) demonstrated various beneficial traits, including salinity tolerance, IAA production, siderophore production, and phosphate solubilization. Inoculation of *P. vulgaris* with *P. orientalis* resulted in significant growth improvements, underscoring its potential as a biofertilizer. Further, rhizobacteria *A. larrymoorei* and *P. orientalis*, showing high chromium tolerance (1840µg/ml), could be a valuable resource for soil bioremediation. *B. cepacian*, with its high salt tolerance (14%), shows promise for improving plant growth under saline conditions. Understanding the mechanisms through which these strains promote growth and development could lead to establishing effective microbial consortia, offering future strategies for advancing sustainable agricultural practices.

**Author's Contributions**

CRD: Conceptualization, designing the work, Supervision, fund arrangement, correction of the manuscript; MT: Experiments, data analysis, original draft of the manuscript.

**Declaration**

There are no conflicts of interest among the authors

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