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RESEARCH ARTICLE

Biopriming of seeds with microbial biostimulant (*Bacillus megaterium*) on improvement of seedling growth, biochemical and root traits of rice (*Oryza sativa* L.)

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Abstract

This study aimed to evaluate the potential of *Bacillus megaterium* in improving seed germination and rice seedling's morpho-physiological and biochemical traits. The experiment was conducted using a completely randomized design (CRD) to evaluate the effect of different concentrations of the biostimulant *B. megaterium* on rice seedlings growth and development under nursery conditions. Biopriming seeds with *B. megaterium* significantly enhanced seed germination and seedling traits, such as seedling shoot and root length, seedling height, number of leaves, seedling vigor, and shoot and root dry biomass, compared to the untreated control. Among the treatments, biopriming seeds with *B. megaterium* at a concentration of 10g kg^{-1} resulted in the greatest improvements across all the recorded parameters. Root traits such as total root length, surface area, average root diameter, root volume, and root tips and forks were also significantly enhanced. Additionally, biochemical changes like total chlorophyll and soluble protein content showed notable improvement in *B. megaterium* biopriming with the concentration of 10g kg^{-1}. Hence, these results suggest that biopriming seeds with *B. megaterium* are an efficient strategy to improve seed germination and shoot and root vigor in rice seedlings.

Keywords

Bacillus megaterium ; biopriming; biostimulant; rice; root traits; seedling vigor

Introduction

Biostimulants are increasingly acknowledged as vital and environmentally friendly elements of sustainable agriculture, providing a feasible substitute for agrochemicals, mitigating environmental damage, eliminating health hazards, and cutting input expenses in crop production. Biostimulants include substances or microorganisms that enhance plant growth, nutrient use efficiency, crop health, and overall crop quality by regulating various physiological and biochemical processes (1, 2). Microbial-based biostimulants, like bacteria (PGPB: plant growth-promoting bacteria) and fungi (PGPF: plant growth-promoting fungi) namely *Bacillus*, *Pseudomonas*, *Azospirillum,* and mycorrhizal fungal species, are commonly applied to crop plants through seed treatment, soil application, or foliar spray (3, 4). Microbial formulations with potent bacterial strains, particularly from the *Bacillus* genus, demonstrate a broad spectrum of plant growth-promoting properties. These include nitrogen fixation, solubilization of essential minerals (phosphorus, potassium, and zinc), production of phytohormones, siderophores, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, antimicrobial substances, volatile organic compounds (VOCs), exopolysaccharides, and hydrolytic enzymes. These properties significantly enhance plant growth, health, and yield by improving nutrient uptake, promoting root development, and enhancing photosynthetic activity and resistance to phytopathogens and environmental stresses (5, 6).

An endophytic bacterial isolate, *Bacillus subtilis*, isolated from sugarcane, was observed to enhance the growth, physiological parameters, and nutrient content of its seedlings by synthesizing growth-promoting compounds, including 6-benzyl adenine, cytokinin (CT), indole-3-acetic acid (IAA), zeatin (ZT), and organic acids (5). Similarly, inoculation with *Bacillus altitudinis* (B197) enhanced maize seedling growth by improving seed germination percentage, shoot and root length, and dry biomass production. This effect was attributed to its ability to produce siderophores and solubilize potash and zinc (7). In mustard, inoculation with phosphate-solubilizing *B. megaterium* significantly enhanced root and shoot growth, chlorophyll content, and metabolites such as glucose, sucrose, fructose, and amino acids by regulating the nutrient uptake that is essential for metabolic activity (8). In *Arabidopsis*, *B. megaterium* was shown to promote lateral root initiation and overall plant growth by regulating auxin biosynthesis and redistribution (9). Outcome of several studies confirmed that the application of bacterial biostimulants (BBs) improves seedling vigor, growth, yield, and quality in various crops, including sugarcane (5), tomato (10), pearl millet (11), wheat (12), barley (13), soybean (14), maize (15), and little millet (16). Besides enhancing plant growth and productivity, BBs prepare plants to endure and withstand abiotic and biotic stresses by eliciting systemic resistance (17-19).

Rice (*Oryza sativa* L.) is a staple food crop that feeds more than half of the world's population. India is the second largest producer of rice, cultivating nearly 45 million hectares of land, with a total grain production of almost 178 million tonnes in 2021, second only to China (20). The growth of rice plants typically progresses through three agronomical phases: vegetative, reproductive, and grain filling and ripening (maturity) (21). From seed germination to panicle initiation, rice undergoes the vegetative phase of development, which significantly influences grain yield. The seed germination potential and seedling vigor of rice are key factors that contribute to subsequent tillering quality, crop growth, and yield (22).

Moreover, the age of the seedling at transplanting is a critical factor, highly correlated with grain yield potential (23). Therefore, improving seed germination and seedling vigor is crucial for improved rice growth and grain yield (21). Interestingly, biopriming seeds with microbial biostimulants, including *Bacillus* sp., significantly improve seed germination, seedling growth, and root vigor (10, 15). Various studies have confirmed that biopriming rice seeds with bacterial inoculants enhance seed germination, seedling growth, and root vigor (24-26). Although considerable progress has been made in identifying efficient microbial strains that can enhance rice growth from seed germination to yield improvement, further research is needed. Moreover, the effect of biostimulants on plant growth and yield depends on the optimal concentration of the products, which is essential to achieve maximum benefits. This study was thus undertaken to assess the dose-response effect of *B. megaterium* (Heiko seed) on seed germination, seedling vigor, root characteristics, and biochemical alterations in rice under nursery settings.

Materials and Methods

The experiment was conducted at the Wetland, Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, in 2023, following a completely randomized design (CRD) with four treatments and six replications. The paddy seeds (variety CO 53) used in the study were purchased from the Department of Rice, Tamil Nadu Agricultural University, Coimbatore. The seeds were then soaked in water overnight and biotized with Heiko seed containing *B. megaterium*. The biostimulant, Heiko seed (*B. megaterium*), used in the study was obtained from M/s. Valagro Bioscience Limited, Hyderabad, India.

Biotization of the seeds was performed by mixing different concentrations of Heiko seed with 1 kg of pre-soaked paddy seeds using 2 % carboxymethyl cellulose as a sticking agent. The experimental treatments included: (T1) untreated control, (T2) seeds biotized with Heiko seed at the rate of 5g kg^{-1} of seeds, (T3) seeds biotized with Heiko seed at the rate of 10g kg^{-1} of seeds, and (T4) seeds biotized with Heiko seed at the rate of 15g kg^{-1} of seeds. After biotization, the seeds were placed in a gunny bag and incubated under dark conditions for 12 h. Following incubation, the pre-germinated biostimulants-treated seeds and untreated control seeds were sown separately in the wet nursery, with a bed size of 1×1 m, pre-fertilized with diammonium phosphate (DAP) at the rate of 200 g sq. m^{-1}. The nursery was irrigated as needed after seed germination. After 18 days of sowing (DOS), rice seedling traits, such as seed germination (%), seedling vigor index, shoot and root length (cm), seedling height (cm), number of leaves per seedling, shoot and root dry biomass (mg $seedling^{-1}$), and root-to-shoot ratio, were recorded in the nursery. Additionally, the normalized difference vegetation index (NDVI) was recorded using a portable GreenSeeker handheld sensor (Trimble) (27).

Root samples from treated and untreated control plants were collected from the experiment and washed thoroughly with distilled water to remove dust and debris adhering to the root surface. The washed root samples were put on a tray with water in it, and they were positioned such that they did not overlap. The tray was then placed in the instrument WinRHIZO dual optical scanner (REGENT STD 4800) attached to the system. The WinRHIZO optical scanner software (version 5.0) was used to acquire root images at a resolution of 400 dpi with a color scale (28). The scanned root images of each treatment were analyzed to measure various root growth and developmental parameters, including total root length (TRL; cm), surface area (SA; cm^2), average diameter (AD; mm), root volume (RV; cm^3), and number of tips (NOT) and forks (NOF).

Finally, leaf total chlorophyll (mg g^{-1} of fresh weight; FW) and soluble protein (mg g^{-1} of FW) content were estimated using the dimethyl sulfoxide (DMSO) and Folin-Ciocalteau reagent methods, respectively (29, 30). About 100 mg of leaf tissue was immersed in a test tube containing 10 ml of DMSO overnight at room temperature to extract the chlorophyll. The extract was then made up to a volume of 25 ml with DMSO. About 3 ml of the chlorophyll extract was taken, and the optical density (OD) was measured at 652 nm using a spectrophotometer (UV-1900i, Shimadzu, UK). The chlorophyll content was calculated using the following equation.

$$\text{Total chlorophyll (mg g}^{-1}\text{ of FW)} = \frac{\text{O.D. at 652}}{34.5 \times W} \times V \text{ mg g}^{-1} \quad \text{........(Eqn. 1)}$$

Where, W - weight of the leaf sample (g); V- volume of supernatant solution made-up; OD-optical density

About 250 mg of leaf sample was macerated with 10 ml of phosphate buffer for leaf soluble protein estimation, followed by centrifuging at 3000 rpm for 10 min. The supernatant was collected, and the volume was adjusted to 25 ml. About 1 ml of the supernatant was mixed with 5 ml of alkaline copper tartrate reagent and 0.5 ml of Folin-Ciocalteau reagent. The OD was measured at 660 nm using a spectrophotometer (UV-1900i, Shimadzu, UK). The soluble protein content was calculated using bovine serum albumin as the standard, and the results were expressed as mg g^{-1} fresh weight.

Statistical analysis

The experimental data were statistically analyzed using Microsoft Excel (version 2010) and SPSS (version 16.0). The experiment followed a CRD with four treatments and six replications. The significant effects of different concentrations of Heiko seed biostimulants on seedlings, root traits, and biochemical parameters were analyzed using one-way ANOVA (analysis of variance). The mean value difference between the treatments was compared using Duncan's multiple range test (DMRT) at the significant p = 0.05 level. Total chlorophyll and soluble protein were visualised using GraphPad Prism (version 8.0.2). The correlation between the variables of assessed seedling growth, biochemical, and root traits influenced by seed biopriming with *B. megaterium* (Heiko seed) was performed using Pearson's correlation test ($p< 0.05$).

Results and Discussion

The growth and yield of rice are predominantly influenced by seed germination and seedling vigor, which contribute to tillering quality, vegetative growth, health, and yield. Moreover, the successful establishment of rice seedlings after transplanting depends on root growth and seedling architecture (22). Microbial biostimulants, especially *Bacillus sp.*, have been demonstrated to improve root vigor, seed germination, and seedling growth in various crops (10, 15). The present study evaluated the impact of the microbial biostimulant *B. megaterium* (Heiko seed) on seed germination, seedling growth, biochemical changes, and root vigor of rice. The results demonstrated that *B. megaterium* positively influenced these parameters, leading to improved seedling establishment in rice.

Numerous studies have demonstrated that applying microbial biostimulants through seed biotization improved seed germination percentage and seedling vigor in various crops such as tomato (10), wheat (12), soybean (14), maize (15), little millet (16), rice (24), etc. In the present study, seed germination percentage was significantly higher in seeds treated with Heiko seed at the rate of 10g kg^{-1} of seeds (T3), recording 98.33% (p = 0.04), compared to other treatments. This result was statistically similar to that of Heiko seed treatment at 15g kg^{-1} (T4), which showed a germination rate of 96.67%, whereas the untreated control (T1) recorded a lower germination rate of 90.83% (Table 1). Similarly, seedlings treated with Heiko seed at 10g kg^{-1} (T3) exhibited significantly higher shoot ($p < 0.001$) and root lengths (p = 0.016) of 36.50 cm and 12.08 cm, respectively, compared to other treatments. The shoot and root lengths of rice seedlings treated with Heiko seed at 10g kg^{-1} (T3) and Heiko seed at 15g kg^{-1} (T4) were statistically similar. In comparison, the untreated control recorded lower values of 25.70 cm and 7.58 cm for shoot and root lengths, respectively (Table 1 and Fig. 1).

Seedling height followed a similar pattern, with the maximum height (48.58 cm) observed in seedlings treated with Heiko seed at 10g kg^{-1} of seeds (T3). This was on par with treatments with Heiko seed at 15g kg^{-1} (T4) and Heiko

Table 1. Effect of microbial biostimulant on seedling traits of rice.

Treatments	Germination %	Shoot length (cm)	Root length (cm)	Seedling height (cm)	Number of leaves	Seedling vigor index	Shoot dry biomass (g)	Root dry biomass (g)
T1	90.83^b	25.70^b	7.58^b	33.28^b	3.00^b	3025.92^c	0.34^b	0.14^b
T2	95.00^{ab}	33.75^a	9.62^{ab}	43.37^a	3.83^a	4133.33^b	0.39^b	0.15^b
T3	98.33^a	36.50^a	12.08^a	48.58^a	4.00^a	4777.83^a	0.48^a	0.18^a
T4	96.67^a	34.22^a	10.63^a	44.85^a	3.83^a	4324.17^{ab}	0.41^{ab}	0.16^b
Mean	95.21	32.54	9.98	42.52	3.67	4065.31	0.41	0.16^b
SE (Standard Error)	2.54	1.58	1.28	2.50	0.31	269.26	3.35	0.01
CD (Critical Difference) (0.05)	5.30*	3.30**	2.68*	5.22**	0.64*	561.568**	0.07**	0.02**

Values are the mean of replicates; Values followed by the same letter are not significantly different from each other as determined by DMRT (p≤0.05). **T1**: Untreated Control; **T2**: Heiko seed at the rate of 5g kg^{-1}; **T3**: Heiko seed at the rate of 10g kg^{-1}; **T4**: Heiko seed at the rate of 15g kg^{-1}

Fig. 1. Effect of microbial biostimulant on growth of rice seedlings. **T1:** Untreated control; **T2:** Heiko seed at the rate of 5g kg^{-1}; **T3:** Heiko seed at the rate of 10g kg^{-1}; **T4:** Heiko seed at the rate of 15g kg^{-1}.

seed at 5g kg^{-1} (T2), while the untreated control recorded the lowest seedling height of 33.28 cm (Table 1; p<0.001). The number of leaves per seedling ranged from 3 to 4, with the Heiko seed treatment at the rate of 10g kg^{-1} of seeds (T3) producing a maximum of 4 leaves (p=0.016), compared to the untreated control, which had only 3 leaves (T1). These results align with previous findings on the beneficial impact of microbial biostimulants, which promote plant growth through mechanisms such as producing plant growth hormones and improved nutrient uptake (12, 14). In addition, it was found that microbial inoculum enhanced seed germination and seedling attributes in crops cultivated in stressed conditions like salinity and drought, besides optimal conditions (16, 19, 24).

Seedling vigor is a key component in improving tillering, growth, and yield of rice (22). In the current study, the seedling vigor index was significantly higher in seedlings treated with Heiko seed at the rate of 10g kg^{-1} (T3), followed by Heiko seed at the rate of 15g kg^{-1} (T4), with values of 4777.83 and 4324.17, respectively (p<0.001). Compared to Heiko seed treatments, the untreated control (T1) recorded the lowest seedling vigor index of 3025.92 (Table 1). Among the treatments, seedlings treated with Heiko seed at the rate of 10g kg^{-1} (T3) recorded significantly higher shoot (p<0.001) and root dry biomass (p<0.001) of 0.48 and 0.18 g $seedling^{-1}$, respectively, while the untreated control (T1) recorded the lowest shoot and root dry biomass of 0.34 and 0.14 g $seedling^{-1}$, respectively (Table 1). Several studies have confirmed the positive effects of microbial biostimulants on seedling vigor and dry biomass in various crops (31, 32).

The balance between root and shoot growth is essential for plant development, as it enables efficient nutrient and water uptake, ultimately influencing crop performance (33). In the present study, the application of Heiko seed at the rate of 10g kg^{-1} (T3) resulted in a significantly higher root-to-shoot ratio of 0.60 compared to the untreated control (T1), which recorded the lowest value of 0.42 (Table 2). The NDVI meter, a common tool used to measure the greenness and vigor of the plants, helps relate plant photosynthetic activity, growth, and health (27), was also notably higher in T3 (0.85), while the untreated control showed the lowest NDVI of 0.73. Heiko seed applied at the rate of 15g kg^{-1} (T4) produced a root-to-shoot ratio of 0.52 and an NDVI of 0.83, while the 5g kg^{-1} treatment (T2) had a root-to-shoot ratio of 0.40 and an NDVI of 0.81 (Table 2).

Table 2. Effect of microbial biostimulant on root shoot ratio and NDVI of rice seedlings.

Treatments	Root shoot ratio	NDVI
T1	0.42^{b}	0.73^{c}
T2	0.40^{b}	0.81^{b}
T3	0.60^{a}	0.85^{a}
T4	0.52^{ab}	0.83^{ab}
Mean	0.49	0.81
SE	0.04	0.01
CD (0.05)	0.12**	0.04**

Values are the mean of replicates; Values followed by the same letter are not significantly different from each other as determined by DMRT (p ≤0.05). **T1**: Untreated Control; **T2**: Heiko seed at the rate of 5g kg^{-1}; **T3**: Heiko seed at the rate of 10g kg^{-1}; **T4**: Heiko seed at the rate of 15g kg^{-1}

These results clearly state that biostimulant treatments enhance root development and overall plant vigor, particularly when applied at 10g kg^{-1} (T3). The improvements in root-to-shoot ratio and NDVI observed in the Heiko seed treatments, especially at the 10g kg^{-1} concentration, suggest that the biostimulant promotes root architecture and shoot growth through enhanced nutrient and water uptake efficiency. The statistical significance of these results is supported by the critical differences (CD) at the 5% level, which were 0.12 for the root-to-shoot ratio and 0.04 for NDVI, respectively (34, 35). The positive effects of Heiko seed on crop performance are likely attributed to its ability to regulate plant physiological processes, particularly through the production of plant growth hormones, which contributes to improved biomass allocation and enhanced shoot vigor (33).

Leaf chlorophyll content and soluble protein are indicators of plant growth, directly correlated with photosynthetic activity, which is essential for understanding the photosynthetic process and assimilating production (36, 37). In the current study, Heiko seed-treated plants recorded higher total chlorophyll and soluble protein content across all Heiko seed concentrations compared to the untreated control ($p<0.001$). However, the highest total chlorophyll content was recorded in the treatment with Heiko seed at the rate of 10g kg^{-1} (T3) with 1.22 mg g^{-1} of fresh weight (FW) leaf tissue, which was statistically on par with Heiko seed at the rate of 15 g kg^{-1} (T4) and Heiko seed at the rate of 5 g kg^{-1} (T2) treatments (1.13 and 1.07 mg g^{-1} of FW, respectively). The total chlorophyll content was lower in the untreated control (T1) with 0.76 mg g^{-1} of FW (Fig. 2A). Similarly, Heiko seed treatments recorded higher soluble protein content compared to the untreated control ($p<0.001$). The maximum soluble protein content of 17.53 mg g^{-1} of FW was recorded in Heiko seed at the rate of 10 g kg^{-1} (T3), while the untreated control (T1) recorded the lowest soluble protein content of 13.54 mg g^{-1} of FW (Fig. 2B). Similar results are also found in other studies (37, 38).

Crop growth depends on its ability to obtain minerals and water from the soil through extensive root system development (33). In the present study, seedlings treated with Heiko seed at the rate of 10 g kg^{-1} (T3) recorded higher total root length (49.63 cm) and root surface area (81.58 cm^2) compared to other treatments. The untreated control (T1) recorded lower total root length and root surface area of 31.35 cm and 70.0 cm^2, respectively (Table 3 and Fig. 3). Similarly, average root diameter and root volume were higher in Heiko seed at the rate of 10 g kg^{-1} (T3) treatment, with values of 1.23 mm and 7.13 cm^3, respectively ($p<0.001$). Among the treatments, the untreated control (T1) recorded the lowest average root diameter of 0.71 cm and root volume of 5.23 cm^3, respectively (Table 3). As expected, the number of root tips and forks was significantly higher in seedlings treated with Heiko seed at the rate of 10g kg^{-1} (T3) among the treatments, recording 280.84 and 285.97, respectively. Compared to Heiko seed treatments, the untreated control (T1) recorded fewer root tips and forks, with values of 240.73 and 234.47 respectively (Table 3). These improved in root growth and architecture due to the application of microbial biostimulants through regulating cell division and differentiation by producing plant growth hormones, which contributes to enhanced shoot growth (34, 35).

The pearson correlation analysis revealed a positive correlation between the variables of seedling growth, biochemical traits, root vigor, and the biopriming effect of rice seeds with *B. megaterium* (Tables 4 and 5). Seed germination percentage (GP; $r^2 = 0.476$), seedling growth traits such as shoot length (SL; $r^2 = 0.661$), root length (RL; $r^2 = 0.498$), seedling height (SH; $r^2 = 0.646$), number of leaves (NOL; $r^2 = 0.478$), seedling vigor index (SVI; $r^2 = 0.657$), shoot dry biomass (SDB; $r^2 = 0.442$), and root dry biomass (RDB; $r^2 = 0.420$)

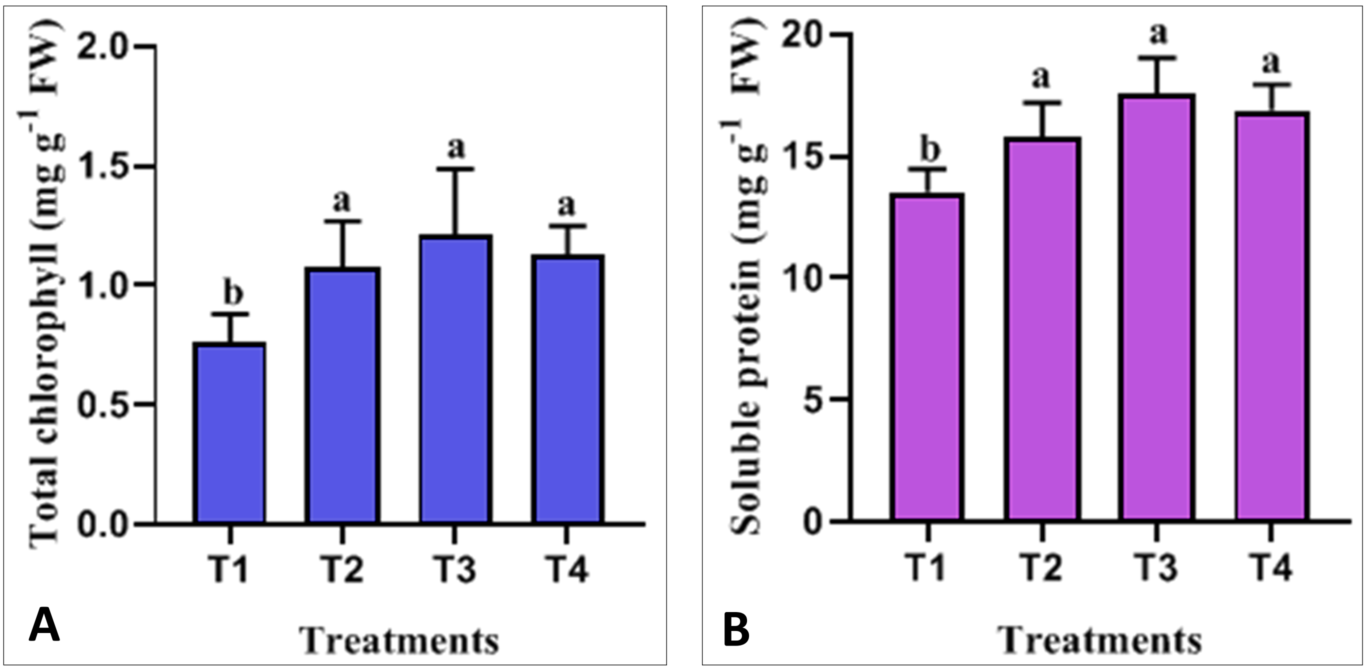

Fig. 2. Effect of microbial biostimulant on total chlorophyll (**A**) and soluble protein (**B**) content of rice seedlings. Values are the mean of replicates; Values followed by the same letter are not significantly different from each other as determined by DMRT ($p \leq 0.05$). **T1:** Untreated control; **T2:** Heiko seed at the rate of 5g kg^{-1}; **T3:** Heiko seed at the rate of 10g kg^{-1}; **T4:** Heiko seed at the rate of 15g kg^{-1}.

Table 3. Effect of microbial biostimulant on root traits of rice seedlings measured by WinRhizo software.

Treatments	Total root length (cm)	Surface area (cm^2)	Average diameter (mm)	Root volume (cm^3)	Number of tips	Number of forks
T1	31.35^{b}	70.07^{c}	0.71^{b}	5.23^{b}	240.73^{b}	235.47^{c}
T2	39.61^{ab}	71.89^{bc}	1.06^{a}	5.53^{b}	259.56^{ab}	250.35^{c}
T3	49.63^{a}	81.58^{a}	1.23^{a}	7.13^{a}	280.84^{a}	285.97^{a}
T4	41.17^{ab}	76.12^{b}	1.11^{a}	6.07^{ab}	272.39^{a}	269.42^{b}
Mean	40.44	74.91	1.03	5.99	263.38	260.30
SEd	4.81	2.30	0.08	0.58	12.31	7.78
CD (0.05)	10.21*	4.87**	0.18**	1.23**	26.09*	16.50**

Values are the mean of replicates; Values followed by the same letter are not significantly different from each other as determined by DMRT ($p \leq 0.05$). **T1**: Untreated Control; **T2**: Heiko seed at the rate of 5g kg^{-1}; **T3**: Heiko seed at the rate of 10g kg^{-1}; **T4**: Heiko seed at the rate of 15g kg^{-1}.

Fig. 3. WinRhizo rice root images of the studied treatments and their effects on root parameters. The measurements of root morphology parameters were taken 18 days after transplantation.

and biochemical changes like total chlorophyll content (Chl; r^2 = 0.602), and soluble protein (SP; r^2 = 0.720) were positively correlated with biopriming of seeds with *B. megaterium* (Heiko seed). Moreover, seedling vigor index had a positive correlation with SL (r^2 = 0.968), RL (r^2 = 0.791), SH (r^2 = 0.970), NOL (r^2 = 0.420), SDB (r^2 = 0.428), RDB (r^2 = 0.613), Chl (r^2 = 0.549) and SP (r^2 = 0.643) (Table 4). Similarly, root traits such as total root length (TRL; r^2 = 0.493), surface area (SA; r^2 = 0.584), average diameter (AD; r^2 = 0.674), root volume (RV; r^2 = 0.444), number of tips (NOT; r^2 = 0.593) and number of forks (NOF; r^2 = 0.713) were had a highly positive correlation with biopriming of seeds with *B. megaterium* (Heiko seed) (Table 5). These results indicated that biopriming of seeds with the microbial biostimulant, *B. megaterium* (Heiko seed) significantly improved seedling growth, biochemical traits, and root characteristics of rice.

Conclusion

Biopriming seeds with microbial biostimulants have been shown to enhance seedling vigor, crop growth, plant health, and yield in both agricultural and horticultural crops. Specifically, the application of *B. megaterium* (Heiko seed) at a concentration of 10g kg^{-1} through seed biopriming significantly improved key growth parameters in rice seedlings, including seed germination, root and shoot development, total chlorophyll content, and soluble protein levels. These enhancements in rice seedling traits could be attributed to microbial inoculation through various plant growth-promoting mechanisms. Therefore, these findings suggest that biopriming seeds with *B. megaterium* (Heiko seed) are an effective strategy for enhancing seed germination and seedling vigor, which could lead to better crop establishment. However, the effectiveness of microbial

Table 4. Correlation of assessed seedling variables with microbial (Heiko seed) biopriming of rice seeds.

Variables	Biostimulant	GP	SL	RL	SH	NOL	SVI	SDB	RDB	Chl	SP
Biostimulant	1	0.476*	0.661**	0.498*	0.646**	0.478*	0.657**	0.442*	0.420*	0.602**	0.720**
GP		1	0.598**	0.232	0.503*	0.091	0.697**	0.305	0.379	0.352	0.453*
SL			1	0.720**	0.965**	0.418*	0.968**	0.386	0.662**	0.564**	0.615**
RL				1	0.876**	0.524**	0.791**	0.360	0.412*	0.429*	0.508*
SH					1	0.487*	0.970**	0.404	0.614**	0.553**	0.619**
NOL						1	0.420*	0.454*	0.207	0.595**	0.505*
SVI							1	0.428*	0.613**	0.549**	0.643**
SDB								1	0.341	0.439*	0.739**
RDB									1	0.574**	0.328
Chl										1	0.500*
SP											1

***** Indicates the correlation is significant at the 0.05 level (2-tailed) and ****** indicates the correlation is significant at the 0.01 level (2-tailed). **GP**: Germination percentage; **SL**: Shoot length; **RL**: Root length; **SH**: Seedling height; **NOL**: Number of leaves; **SVI**: Seedling vigor index; **SDB**: Shoot dry biomass; **RDB**: Root dry biomass; **Chl**: Total chlorophyll content; **SP**: Soluble protein.

Table 5. Correlation of assessed seedling variables with microbial (Heiko seed) biopriming of rice seeds.

Variables	Biostimulant	TRL	SA	AD	RV	NOT	NOF
Biostimulant	1	0.493*	0.584**	0.674**	0.444*	0.593**	0.713**
TRL		1	0.669**	0.644**	0.444*	0.392	0.637**
SA			1	0.568**	0.645**	0.373	0.698**
AD				1	0.567**	0.515*	0.652**
RV					1	0.599**	0.687**
NOT						1	0.851**
NOF							1

***** Indicates the correlation is significant at the 0.05 level (2-tailed) and ****** indicates the correlation is significant at the 0.01 level (2-tailed). **TRL**: Total root length; **SA**: Surface area; **AD**: Average diameter; **RV**: Root volume; **NOT**: Number of tips; **NOF**: Number of forks.

biopriming depends on several factors, such as the efficiency of the microbial culture, the concentration of the inoculum used, and environmental factors like soil physiochemical properties. Plant species characteristics, including growth stages, morpho-physiological traits, and metabolic properties, also play a role. Hence, the development of inoculants with location-and crop-specific isolates, along with the standardization of optimum concentrations of products, is essential for achieving significant benefits. Further research is needed to explore the long-term effects of microbial biopriming across multiple growing seasons and in varying agro-climatic zones. Moreover, large-scale field trials are necessary to confirm the efficacy of microbial biostimulants in improving crop growth and yield.

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Authors' contributions

DV and VR conceptualized and conducted the study. VR, NS and GPK validated the data. DV and SA performed the statistical analysis, data visualization, drafting of original manuscript. VR, NS and MRL contributed to the review and editing of the final manuscript. All co-authors reviewed the final version and approved the manuscript for submission.

Compliance with ethical standards

Conflict of interest: All authors do not have any conflict of interest to declare.

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