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Induction of Root Growth and Suppression of Sheath Blight Disease in Paddy (*Oryza sativa* L.) by Endophytic Bacteria

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In this study, three endophytic bacteria including two Gram +ve and one Gram -ve, were evaluated for suppression of sheath blight disease caused by *Rhizoctonia solani* in paddy. The endophytic bacteria tested were *Bacillus subtilis* strain TRO4, *B. subtilis* strain CLO5 and *Pseudomonas cichorii* strain TLO7. The experiments were conducted under pot culture conditions in paddy var. MTU-7029 during *Kharif* 2021 and the treatments were: T1- un-inoculated (control), T2- pre-challenged in soil with the pathogen (*R. solani*), T3- pathogen pre-challenged + seed treatment with *B. subtilis* strain TRO4, T4- pathogen pre-challenged + seed treatment with *B. subtilis* strain CLO5 and T5- pathogen pre-challenged + seed treatment with *P. cichorii* strain TLO7 and T6- pathogen pre-challenged + Carbendazim (2 g/kg). There were four replications. The observations such as seed germination, disease occurrence, plant growth, root architecture, chlorophyll content and peroxidase activity in leaf tissues were recorded. The results showed the endophytic bacteria were found to be effective in enhancing the plant growth and reducing disease occurrence at par with chemical fungicide. The root growth parameters were found to be higher in pathogen pre-challenged and seeds treated with *B. subtilis* CLO5 (T4). The average number of lesions/plant and length of lesions (cm)/plant was found lower in (T4) and the corresponding value was 2. Considering the parameters of enhanced seed germination, induction of root growth and suppression of sheath blight disease, the seed priming with endophytic bacteria, *B. subtilis* strain CLO5 is a promising strategy for Sheath blight disease management in paddy.

Introduction

Rice is an important staple food grain. In India, rice production recorded was 137.8 million tons in the year 2023-24 (Source: Press Information Bureau, Government of India). Among various fungal diseases affecting rice, sheath blight is one of the most destructive across the world, caused by the fungal pathogen *Rhizoctonia solani* (Nagendran *et al.,* 2014). Sheath blight disease of rice is a major production constraint, causing an annual yield loss up to 40-50%, (Jamali *et al.,* 2019; Sunera *et al.,* 2024). Along with rice crops, *R. solani* can also infect a wide range of host plants (barley, lettuce, maize, tomato, and sorghum), resulting in grave yield loss under favorable conditions.

The sclerotia of *R. solani* can survive in the soil for more than 2 years and are easily spread during field preparation and flooding. The typical visible disease symptoms of *R. solani* are the formation of gray to dark brownish margin colors with oval to irregular lesions on rice sheath and leaf blade, plant lodging, and the presence of empty grains (Singh and Sinha, 2009).

Among the different sheath blight disease management strategies, bio-control is found to be environment-friendly and cost-effective approach that can be substituted with chemicals to achieve a greater level of crop protection and sustained yields by avoiding the development of resistance by the fungal pathogen. In recent years, the bacterial endophytes receive more attention for biocontrol of plant diseases (Ryan *et al.,* 2007).

Bacterial endophytes are a group of bacteria that have special ability to colonize and reside inside the plants and protect the host plant from biotic and abiotic stresses (Santos *et al.,* 2018). Bacterial endophytes colonize the same niche as plant pathogens and suppress the pathogen by various mechanisms like production of Indole Acetic-Acid (IAA), hydrogen cyanide (HCN), siderophore, hydrolytic enzymes, antibiotics, and induction of systemic resistance (Mageshwaran *et al.,* 2022a). Thus, bacterial endophytes could be used as an efficient bio-control agent against sheath blight in rice as they provide localized protection to the host plant.

Among the bacterial endophytes, Bacillus and Pseudomonas strains have simple nutritional requirements, excellent root colonizing ability and induce the expression of defense proteins upon infections by pathogens (Singh and Sinha, 2009; Nagendran *et al.,* 2014; Kumar *et al.,* 2020).

Recently, Ali *et al.,* (2024); Rabbee *et al.,* (2024) highlighted the importance of discovery of endophytic bacteria with biocontrol and plant growth promoting for developing sustainable agriculture. Thus, the present investigation was aimed to investigate the effect of three endophytic bacteria on induction of root growth and bio-control of sheath blight disease caused by *R. solani* in rice.

Materials and Methods

Microorganisms

The test fungal pathogen, *R. solani* was obtained from Plant-Microbe Interaction Laboratory and Rhizosphere Biology Lab, ICAR-NBAIM, Mau and the endophytic bacteria *B. subtilis* strain TRO4 (isolated from tomato root), *B. subtilis* strain CLO5 (isolated from chickpea leaf) and *P. cichorii* strain TLO7 (tomato leaf) were obtained from Microbial Technology Laboratory, ICAR-NBAIM, Mau and their respective NAIMCC accessions numbers are B-02794, B-02790 and B-02799. The fungal culture and bacterial cultures were grown on the Petri plates containing potato dextrose agar (PDA) and nutrient agar (NA) (Himedia Pvt. Ltd., Mumbai, India), respectively. The cultures were grown at 28 ± 2 °C for 7 days (fungus) and 2 days (bacteria). The culture plates were preserved at 4 °C till further use.

Antagonistic assay

The antagonistic activity of endophytic bacteria was tested by dual culture plate assay (Mageshwaran *et al.,* 2022b). Briefly, the fungal disc (5mm diameter) was made by unplugging the fully grown fungal pathogen in PDA plate using the flame sterilized cork borer. The fungal disc was kept at the center of a fresh PDA plate and bacterial endophyte was streaked on both sides of the Petri plates equidistant from the disc and incubated at 28 ± 2°C for 48 hours. The inhibition percentage was calculated using the formula: Percent inhibition (PI) = (A-B)/A × 100, where A is the diameter of mycelial growth of the fungal pathogen in the control plate, B is the diameter of mycelial growth of fungal pathogen in the dual culture plate.

Determination of IAA production

The IAA production by endophytic bacteria (TRO4, CLO5 and TLO7) was determined as described by Lebrazi *et al.,* (2020). Briefly, the bacteria were inoculated in 5 mL of NB containing 100 ppm of L-tryptophan and incubated at 28 ± 2 °C for 48 hours at a shaking speed of 150 rpm in an orbital shaking incubator. Afterward, bacterial cultures were centrifuged at 10.000 rpm for 10 min at 4 °C. Two mL of supernatant was mixed with 4 mL of Salkowski reagent (comprising 2 mL of 0.5 M FeCl3 in 49 mL water and 49 mL of 70 % Perchloric acid ($HClO_4$)). The mix was incubated for 20 min in the dark at 28 ± 2 °C, and then absorbance was measured at 530 nm. The concentration of IAA produced was estimated using a standard IAA curve.

Pot culture experiment

The endophytic *Bacillus subtilis* strains (TRO4, CLO5 and TLO7) were evaluated for growth and antagonism against sheath blight disease in paddy (var. MTU 7029). Pot culture experiments were conducted from July to November 2021 (*Kharif* season) at ICAR-NBAIM, Mau.

Preparation of inoculum

The inoculum of *R. solani* was developed by inoculating the mother culture in a flask containing the autoclaved (two tomes) sand and maize grains at 8:1 (w/v) according to Rudresh *et al.,* (2005) with a slight modification in that maize grains were used in place of sorghum. The inoculated flasks were incubated for 2 weeks at 28 ± 2 °C and used for inoculating soil. The inoculum of endophytic bacteria was prepared by inoculating the culture (from agar plates) to the flask containing 100 ml of sterilized nutrient broth (1X) and incubated for 24 h under shaking conditions (125 rpm) at 28 ± 2 °C. The colony-forming unit of the inoculum was calculated as 1×10^8 CFU ml^{-1}.

Experimental set-up

The experimental soil was collected from the agricultural farm, ICAR-NBAIM, Mau. The physico-chemical properties of the soil used in the experiment were pH (8.0), electrical conductivity (0.81 dS/m), and organic carbon (0.39 %). The experimental soil was moistened and autoclaved twice at 12 h intervals. Clean plastic pots (9 × 15 cm) were three-quarters filled with sterile soil. Each pot contained 2 kg of sterile soil. The fungal inoculum (10 g) with a colony forming unit of 1×10^4 CFU g^{-1} was added to the pots and mixed thoroughly. The inoculated pots were incubated for one week to develop and establish the fungal inoculum in the pots containing experimental soil (Rudresh *et al.,* 2005). Seed priming with endophytes was done by suspending the surface sterilized paddy seeds in 24 hours old endophytes inoculum for 30 min before sowing.

The set of treatments in the experimental study is given in Table 1. A negative control [Uninoculated Control (UC)] was maintained in which neither pathogen nor endophyte was inoculated. Plants were irrigated with Jenson’s liquid nutrient medium (Senthilkumar *et al.,* 2009) once a week for 45 days. Four replications were maintained for each treatment. The experiment was conducted in a completely randomized design (CRD).

Assessment of disease incidence and plant growth

Germination (%) was recorded 7 days after sowing. The plants were observed for the appearance of disease symptoms at 45 days after sowing (DAS). The disease incidence was recorded according to Singh and Sinha (2009) by measuring the number of lesions and length of lesion (cm) per plant. The percent reduction of lesion length (LL) ((LL in Pathogen control – LL in Treated) / LL in Pathogen control × 100) was determined as described by Zheng *et al.,* (2021). At 45 DAS, the plants were uprooted and the roots were washed using slow-running tap water and the observations such as root and shoot length (cm) were made, chlorophyll a and b content (mg/ g) and peroxidase activity in the plant leaf tissue was determined according to the method described by Sadasivam and Manickam (1996).

Analysis of root morphology

Forty-five days after sowing, the plants were uprooted and washed, and cleaned roots were used to study the root architecture. The root parameters such as root length (mm), surface area (cm^2), the average diameter (mm), and root volume (cm^3) were determined using a Hewlett Packard scanner and analyzed using the WinRHIZO V. 2002\u00B0C software (Regent Instuments Inc. Ltd. Quebec, Canada) according to Singh *et al.,* (2017).

Statistical analysis

The data collected in this study were analyzed in a completely randomized design (CRD) using a one-way analysis of variance (ANOVA; WASP.1; ICAR research complex, Goa). For all analyses, the differences were considered to be significant at $p \leq 0.05$.

Results and Discussion

Antagonistic activity

Bacterial endophytes are considered as potential candidates of biocontrol as they reside in the immediate vicinity of the invasive pathogens and compete with the pathogens for nutrients and space. Previously, the biocontrol potential of endophytic bacteria, *B. subtilis* TRO4 and CLO5 against wilt complex pathogens, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Fusarium oxysporum* f. sp. *ciceri* in chickpea has been reported (Mageshwaran *et al.,* 2022a, b). In this study, three endophytic bacteria viz., *B. subtilis* strains TRO4 and CLO5 and *Pseudomonas cichorii* TLO7 were evaluated for biocontrol activity against *R. solani* causing Sheath Blight disease in Paddy.

The antagonistic assay of bacteria against *R. solani* was performed by Dual Culture Assay by calculation of Percent Inhibition (PI). The antagonistic property of endophytic bacteria is depicted in Fig. 1. The PI of *R. solani* by endophytic bacteria TRO4, CLO5 and TLO7 were 58.9, 64.3 and 69.5, respectively (Fig. 2). The plant growth promoting trait was evaluated by estimation of IAA produced by endophytic bacteria. The results showed, the IAA production (µg/mL) was maximum in TLO7 (60.83) followed by TRO4 (5.66) and CLO5 (2.83) (Fig. 2). Among the different plant growth promoting activity, IAA production by biocontrol agents is considered as an important parameter as the possible role of IAA in plant growth promotion, root architecture and strengthening plant immune responses to suppress soil borne diseases (Cui *et al.,* 2024; Wang *et al.,* 2025).

Plant Growth and Biocontrol activity

Researchers attempted to isolate the bacterial endophytes from rice itself (Kumar *et al.,* 2024) as well as from other plants like holy basil (Sahu *et al.,* 2020), *Fraxinus hupehensis* (Zheng *et al.,* 2021) to evaluate the biocontrol activity against sheath blight in rice. In this study, the effect of endophytic bacteria isolated from chickpea leaf (*B. subtilis* CLO5) and tomato root (*B. subtilis* TRO4) and tomato leaf (*P. cichorii* TLO7) on plant growth parameters and biocontrol activity against *R. solani* was evaluated under pot culture study (Fig. 3).

The set of treatments and the pots used in the study is depicted in Fig. 3A while the sheath blight symptom occurred during the study is shown in Fig. 3B. The results of the effect of endophytic bacteria on plant growth parameters and biocontrol activity of rice plants pre-challenged with pathogen, *R. solani* is presented in Table 2. The germination percentage was higher (85 to 88 %) in pathogen pre-challenged soil and endophytic bacteria treated seeds (T3, T4 and T5) followed by only pathogen pre-challenged soil (T2) with the tune of 60 %. The germination percentage was least recorded in T1 and T6 which corresponds to 38 and 35 %, respectively. The plant growth parameters such as shoot and root length, Chlorophyll (Chl a and Chl b) content and biocontrol activity parameters such as peroxidase activity, number and length of lesions were recorded.

The shoot length was recorded higher (36 cm) in pathogen pre-challenged and fungicide treated seed (T6) and lower in only pathogen pred-challenged soil T2 (23.9 cm). The root length was recorded maximum (17.3 cm) in pathogen pre-challenged and fungicide treated seed (T6) and lower in only pathogen pre-challenged soil T2 (12.6 cm). The chlorophyll a and b (mg/g) were higher in pathogen pre-challenged and fungicide treated seed (T6) and the corresponding values were 1.68 and 0.31, respectively.

The chlorophyll a and b (mg/g) were lower in only pathogen pre-challenged soil and the corresponding values were 0.94 and 0.17, respectively. The peroxidase activity (U/g) was found higher (2.16) in pathogen pre-challenged and CLO5 treated seed (T4) and lower (1.15) in un-inoculated control (T1). The average number of lesions/plant and length of lesions/plant was found higher (6 and 8, respectively) in only pathogen pre-challenged soil (T2) while the lowest was recorded in pathogen pre-challenged soil and CLO5 treated seed (T4) and the corresponding value was 2. In a similar study, the endophytic bacterium, *B. velezensis* LS123N was found to be a promising strain for biocontrol of rice diseases including bacterial blight, bakane disease, phythium disease and brown spot disease. The number of blight lesions and the length of lesions was significantly lower in rice seeds treated with endophytic bacterium, *B. velezensis* LS123N (Tu *et al.,* 2024). The sheath blight incidence in rice plants was lower in seeds treated with endophytic *B. amyloliquefaciens* B-19 (7.2 %) and at par with chemical treatment (0.1 % propicanazole).

Besides, the root and shoot length, chlorophyll content, peroxidase activity, phenol and proline content were higher in endophytic bacteria treated rice plants (Sunera *et al.,* 2024).

The endophytic bacterium, *B. velezensis* D61-A showed 71 % reduction in sheath blight lesion length in rice plants (Zheng *et al.,* 2021). The results are in accordance with our study in which *B. subtilis* CLO5 treated rice plants showed 75 % reduction of lesion length compared to pathogen control.

Table.1 Treatments detail

T1	**un-inoculated (control)**
T2	pre-inoculated in soil with the pathogen (*R. solani*)
T3	pathogen pre-challenged + seed treatment with *B. subtilis* strain TRO4
T4	pathogen pre-challenged + seed treatment with *B. subtilis* strain CLO5
T5	pathogen pre-challenged + seed treatment with *P. cichorii* strain TLO7
T6	pathogen pre-challenged + Seed treatment with Carbendazim (@2 g/kg of seed)

Table.2 Effect of endophytic bacteria inoculation in rice plants pre-challenged with *R. solani*

Treat-ments	**Plant growth property**					**Bio-control activity**			
	Germination (%)	Shoot length (cm)	Root length (cm)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Peroxidase (Units/g)	Average No. of lesions per plant	Average Length of lesion (cm) per plant	Percent reduction of lesion length
T1	38^{C}	25.3^{c}	15.3^{b}	0.94^{d}	0.17^{d}	1.15^{e}	0	0	-
T2	60^{b}	24.9^{c}	12.6^{c}	1.28^{c}	0.24^{bc}	1.47^{d}	6	8	-
T3	88^{a}	30.8^{b}	15.6^{b}	1.47^{b}	0.27^{ab}	2.01^{b}	2	3	62.5
T4	85^{a}	31.0^{b}	16.3^{bc}	1.38^{bc}	0.21^{cd}	2.16^{a}	2	2	75
T5	88^{a}	32.0^{b}	16.1^{c}	1.34^{c}	0.25^{bc}	1.03^{f}	3	4	50
T6	35^{c}	36.0^{a}	17.3^{a}	1.68^{a}	0.31^{a}	1.68^{c}	3	3	62.5
CV	5.58	6.09	5.64	4.5	13.79	3.09	-	-	-

Note: CV: Co-efficient of Variation; The treatments detail is given in Table 1.

Table.3 Effect of bacterial endophytes inoculation on root parameters of rice plants pre-challenged with *R. solani*

Treatments	**Root length (mm)**	**Surface area (cm^2)**	**Average root dia (mm)**	**Root volume (cm^3)**
T1	188.28	66.76	1.13	1.88
T2	239.67	59.60	0.79	1.18
T3	276.40	76.24	0.88	1.67
T4	389.54	122.31	1.00	3.06
T5	319.44	103.38	1.03	2.66
T6	300.20	92.17	0.98	2.25

Note: The treatments detail is given in Table 1.

Figure.1 Antagonistic property of endophytic bacteria against *R. solani*
(A) *R. solani* control, (B) *R. solani* vs. TRO4, (C) *R. solani* vs. CLO5, (D) *R. solani* vs. TLO7

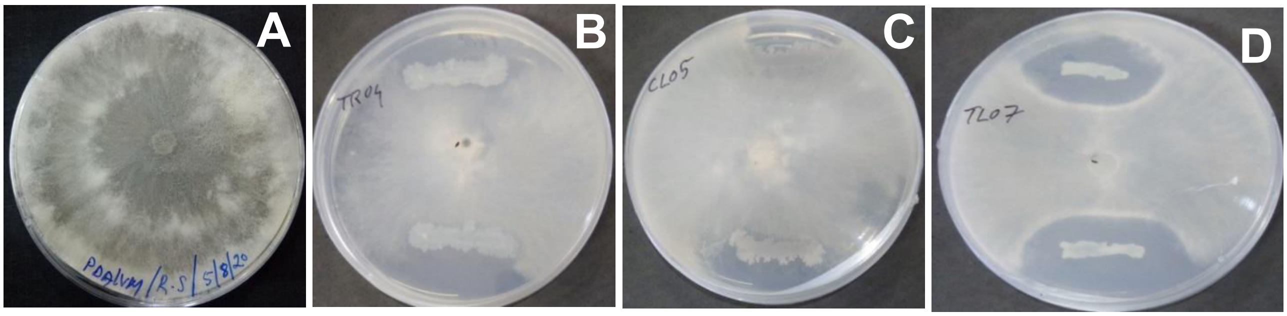


Figure.2 Percent Inhibition (PI) of *R. solani* and Indole Acetic Acid (IAA) production by endophytic bacteria

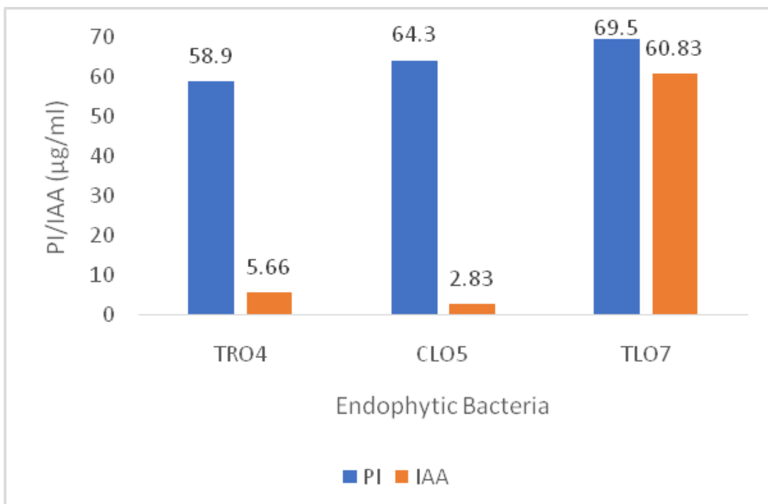
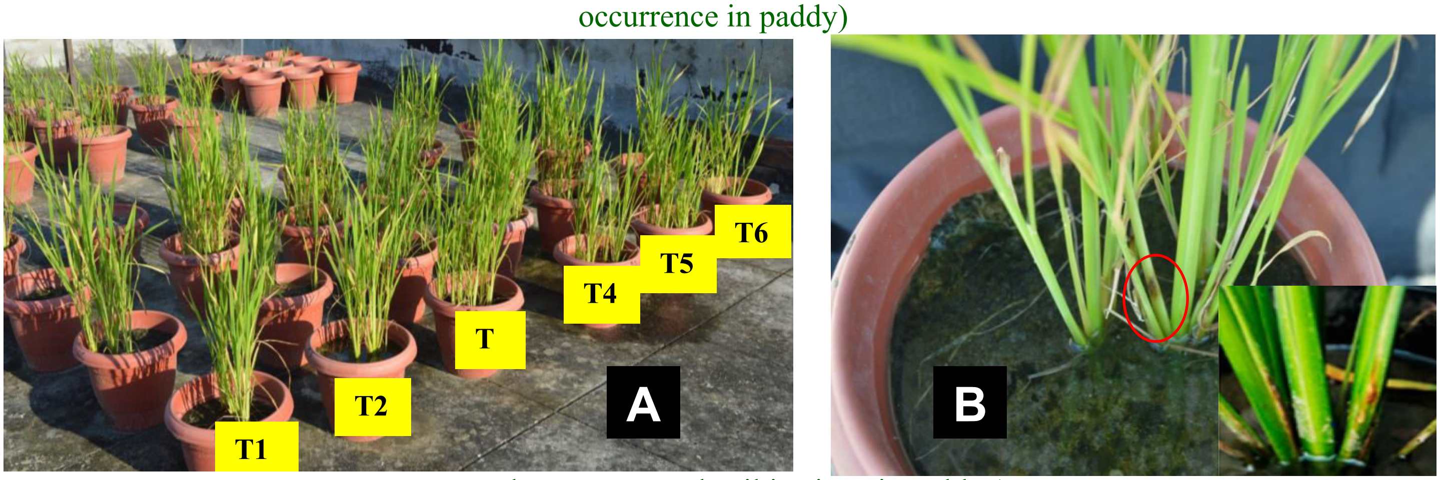
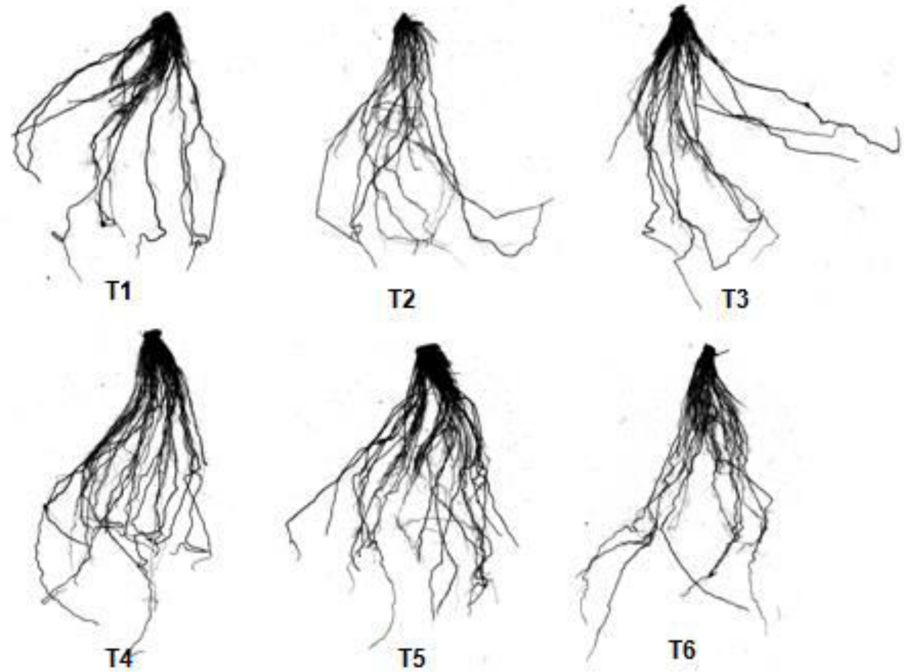


Figure.3 Pot culture study (A- Experimental set up; B- The red circle indicates the sheath blight disease occurrence in paddy)



Note: The treatments detail is given in Table 1.

Figure.4 Effect of endophytic bacteria inoculation on root architecture of rice plants pre-challenged with *R. solani.*



Note: The treatments detail is given in Table 1.

Root architecture

The robust root architecture is an important parameter of the crop to overcome biotic and abiotic stresses (Wang *et al.,* 2025). The beneficial traits of endophytic *Bacillus* strains such as IAA production, siderophore, phosphate and potassium mineralization, antimicrobial peptide synthesis etc. play a vital role in root growth and bio-control of bacterial wilt in Ginger (Cui *et al.,* 2024). In this study, the root architecture and parameters were studied using the root scanner. The root architecture of rice plants with the age of 45 DAS is depicted in Fig. 4.

The results of the different toot parameters are presented in Table 3. The root length (mm) was recorded higher (389.54) in pathogen pre-challenged soil and CLO5 treated seed (T4) and lower (188.28) in un-inoculated control (T1). The surface area (cm^2) was found higher (122.31) in pathogen pre-challenged soil and CLO5 treated seed (T4) and lower in un-inoculated control (T1). The average root diameter (mm) was found higher (1.13) in un-inoculated control (T1) and lower (0.79) in only pathogen pre-challenged soil (T2). The root volume (cm^3) was recorded higher (3.06) in pathogen pre-challenged soil and CLO5 treated seed (T4) and lower (1.18) in only pathogen pre-challenged soil (T2).

To conclude, the endophytic bacteria viz., *B. subtilis* strains TRO4 and CLO5 and *P. cichorii* strain TLO7 were found to be antagonistic against the pathogen, *R. solani* (a causative agent of sheath blight in paddy). All the three strains showed more than 50 % inhibition against *R. solani*. The strain(s) have the capability to produce IAA with maximum of 60.83 by *P. cichorii* TLO7. Under, *in vivo* conditions, the endophytic bacteria have the ability to control sheath blight incidence at par with the chemical treatment. Among the endophytic bacteria, *B. subtilis* CLO5 showed higher (75 %) reduction of blight lesion length in rice plants compared to pathogen control. The root analysis showed that the seed priming with *B. subtilis* CLO5 induce the root architecture in terms of root length, surface area and root volume. Thus, the study highlights that the bacterial endophyte, *B. subtilis* CLO5 is a potential candidate for induction of root growth and suppression of Sheath blight in rice.

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Author Contributions

Vellaichamy Mageshwaran: Investigation, formal analysis, writing—original draft.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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