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Assessment of Endophyte Biopriming on plant growth and seed yield under field condition in rice (*Oryza sativa* L.)

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ABSTRACT

Producing quality seeds is hampered by environmental stresses. It is well known that plant endophytes can influence plant growth and development. A field experiment was carried out in three different environments: normal, drought (controlled irrigation), and a saline soil patch to know the impact of endophyte bio-priming on rice crop performance using five fungal endophytes, Viz, LAS6 (Chaetomium sp.), PJ9 (Fusarium sp.), SF5 (Fusarium sp.), V4J (Botryospharia dothedia), V6 E (Fusarium sp.), and one bacterial endophyte, Bacillus amyloliquefaciens (BA). Most of the growth traits investigated exhibited non-significant results due to treatments. The endophyte LAS 6 recorded the minimum number of days (3) to seedling emergence, the endophyte PJ 9 recorded the maximum plant height (88.2 cm) at 90 DAS, and the endophyte V4 J recorded the greater number of tillers per plant (14.7). Under drought conditions, it was shown that the number of tillers per plant was significant, with endophyte PJ 9 recording more tillers per plant (14.4). It was shown that the seed yield characteristics were statistically insignificant. However, in contrast to the control, endophyte-treated plants had higher values. In the saline soil patch condition compared to the control, the endophyte-treated plants recorded greater values. The endophyte V4 J measured the highest plant height at 45 days after transplantation (46.9 cm), whereas plants treated with PJ 9 reached their maximum plant height (84.5 cm) at 90 days following transplanting. The endophyte SF 5 recorded the highest values for the traits number of tillers per plant (16.3) and seed yield per ha (3090.5 kg/ha). It's possible that the lack of significance in field conditions is because the soil isn't always homogenous in its microbial diversity. The interactions between soil microbiota and treated endophytes are very important for determining how well treated endophytes can colonize the treated plants.

Key words: Endophyte, Plant growth, Abiotic stress, Biopriming and Rice

INTRODUCTION

Various agricultural issues are now being solved through seed-based technologies. The effectiveness of field and captive breeding programmes heavily influences the "lab-to-farm method" for crop development that is promoted by the agricultural sciences. To improve the growth of seedlings and the establishment of the crop, seed-enabled technologies such as innovative seed improvements and seed treatment methods like priming, pelleting, coating, synthetic seeds, etc. are used.Microorganisms have long been used to enhance the performance of agricultural crops. However, the specifics of how microbes interact with plants to increase desired features have only just become clear to us (van der Heijden and Hartmann, 2016; Muller *et al.*, 2016). However, the decision about how and when these microorganisms are absorbed into the functional plant microbiome is ultimately made by the plant. According to Lareen *et al.* (2016), plant microbiome interaction is an unpredictable mechanism, and the knowledge currently available is insufficient to fully understand it (Berendsen *et al.*, 2012; Ghosh *et al.*, 2020). The unpredictable results of utilizing microorganisms in the field due to this lack of precise understanding have decreased end-user adoption of the technology. Different plant growth-promoting microorganisms have been utilized for many years in biological seed improvements. Plant endophytes are gaining popularity in agricultural research as one of many plants' growth-promoting agents because they have demonstrated success in improving seed quality, plant growth, and development, especially early seedling vigour. These endophytes can be exploited as seed bio-priming agents because of their capacity to symbiotically colonize a variety of plant host systems. The bio-priming method combines biological and physiological elements to safeguard the seed and encourage growth (Afzal *et al.*, 2016; Baradwal *et al.*, 2022).

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Rice is the most important food crop grown around the world, and due to the ever-growing population and climate change, the pressure on the production system with available resources has become a challenging task in agricultural science. The major rice production area is dependent on water availability, particularly canal irrigation. Extensive cultivation of rice under lowland conditions has posed secondary salinization problems in the soil and made it saline. Due to the scarcity of water in agriculture, direct-seeded or aerobic rice cultivation is gaining momentum. In this context, the use of endophytes to make crop systems more tolerant to abiotic stress, particularly salinity and drought, has become one of the research interests in agricultural science in developing sustainable agricultural production technology. In the present study, an attempt has been made to evaluate the efficacy of endophytic microbes in enhancing plant growth and seed yield parameters through the seed bio-priming technique in rice (*Oryza sativa* L.) under abiotic stress conditions.

MATERIAL AND METHODS

The main aim of the experiment was to assess the relevance of seed bio-priming with endophytes to improve crop performance in rice. Field experiments were conducted at Agricultural Research Station (ARS), Gangavathi and Farmers' field at Gejjagaraguppe Village, Magadi Taluk and Ramanagaram District.

Endophyte isolates

Five fungal endophytes and one bacterial endophyte isolate were collected from the School of Ecology and Conservation, UAS, GKVK, Bengaluru. The field experiments were set up in three different conditions viz., normal condition (without stress), saline soil patch and drought stress (controlled irrigation). Experiments under normal and drought conditions were conducted in upland condition (direct seeded) and a saline soil patch was conducted in lowland condition (transplanted) with three replications in Randomized complete block design (RCBD).

Treatments details

T_1	Control	T_4	SF 5 (*Fusarium* sp.)
T_2	LAS 6 (*Chaetomium* sp.)	T_5	V4 J (*Botryospharia dothedia*)
T_3	PJ 9 (*Fusarium* sp.)	T_6	V6 E (*Fusarium* sp.)
T_7	BA (*Bacillus amyloliquefaciens*)		

Drought imposition under field condition

The experiment was conducted at a farmers' field in Gejjagaraguppe village, Magadi taluk, Ramanagara district of Karnataka. To avoid the occurrence of rainy days during the experimentation, the experiment was conducted during the rabi-summer season of the year 2020. Drought was imposed by controlled irrigation; in this experiment, the interval of irrigation was set at 8 days. During the entire crop cycle, 11 irrigations were given. Whereas in normal growth conditions, irrigation was given at 5-day intervals, with 18 irrigations in total.

Experiment under salinity conditions

The salinity experiment was conducted at the Agricultural Research Station (ARS), Gangavathi, UAS, Raichur. The field selected was under secondary soil salinization as these rice fields were irrigated with Tungabhadra canal irrigation. In these fields, a uniform salinity level was not found, and it was a salinity patch with different salinity levels. The electrical conductivity of the soil ranges from 2.43 to 3.38 dSm^{-1} and the pH ranges from 8.2 to 8.5.

Preparation of endophyte inoculums

A single hyphal tip from the actively growing endophyte fungi was cultured aseptically on PDA. five-day-old colony culture was used to prepare mycelial suspension (Dhingra and Sinclair, 1993). The mycelial suspension was prepared by washing the mycelial mat with sterile distilled water using a camel hairbrush. Spores/colony-forming units in the inoculum were counted using a haemocytometer under a light microscope.

Further, the suspension was adjusted to mycelial Colony Forming Units (2×10^6 spore/mycelia ml^{-1}) and used for cross- inoculation.

Preparation Bacterial endophytic suspension

Bacterial endophyte *Bacillus amyloliquefaciens* suspension was prepared by growing bacteria on a nutrient agar plate for 24 h. Further, the bacterial culture colony was transferred into the nutrient broth and allowed to grow for 24 hr. The turbidity was measured to quantify the colony-forming unit and fixed at 2 x 10^6 CFU/ml.

Observations recorded

Days to field emergence, Percent field emergence, Plant height 45 and 90 days after sowing (DAS), SPAD reading, Yield-related parameter like, Number of panicles, Length of panicle, Number of branches per panicle, Number of seeds per panicle, Seed yield per hectare and Harvest index.

Statistical design and analysis

Randomized complete block design (RCBD) and DMRT analysis were done using R-software.

RESULTS AND DISCUSSION

Effect of endophyte bio-priming on growth parameters in rice var. IR 64 under normal and drought conditions

Under normal conditions, most of the growth characteristics studied under field conditions showed non-significant differences due to treatments. However, days to seedling emergence, plant height at 90 DAS, and number of tillers were found to be significant (Table 4.3.1).

Table 1: Effect of seed bio-priming with endophytes on plant growth parameter of rice var. IR 64 under normal and drought condition

Treat-ments	Normal condition						Drought condition					
	Days to seedling emergence	Seedling emergence (%)	Plant height (cm)		SPAD reading	No. of tillers/ plants	Days to seedling emergence	Seedling emergence (%)	Plant height (cm)		SPAD reading	Number of tillers/ plants
			45 DAS	90 DAS					60 DAS	90 DAS		
Control	5.3^{a}	$88\ (9.4)^{a}$	47.6^{a}	81.9^{bc}	33.7^{a}	12.5^{c}	4.3^{a}	9.2^{a}	51.5^{a}	74.9^{a}	32.7^{a}	12.1^{c}
LAS-6	3.0^{b}	$94\ (9.7)^{a}$	51.4^{a}	82.7^{abc}	30.8^{a}	13.9^{ab}	3.6^{a}	9.5^{a}	52.0^{a}	76.3^{a}	33.5^{a}	14.4^{a}
PJ 9	3.6^{ab}	$95\ (9.8)^{a}$	54.5^{a}	88.2^{a}	31.4^{a}	13.3^{bc}	3.6^{a}	9.5^{a}	52.2^{a}	72.2^{a}	30.5^{a}	14.3^{a}
SF 5	3.3^{ab}	$94\ (9.8)^{a}$	48.6^{a}	77.9^{c}	32.6^{a}	12.9^{bc}	3.6^{a}	9.6^{a}	49.2^{a}	76.4^{a}	32.3^{a}	14.2^{a}
V4 J	4.0^{ab}	$93\ (9.7)^{a}$	47.6^{a}	87.1^{ab}	34.3^{a}	14.7^{a}	3.3^{a}	9.5^{a}	50.9^{a}	76.8^{a}	33.8^{a}	13.1^{bc}
V6 E	4.0^{ab}	$94\ (9.7)^{a}$	53.5^{a}	86.9^{ab}	34.5^{a}	13.8^{ab}	4.0^{a}	9.6^{a}	51.6^{a}	77.6^{a}	33.7^{a}	13.6^{ab}
BA	4.6^{ab}	$92\ (9.6)^{a}$	52.5^{a}	77.0^{c}	35.2^{a}	13.0^{bc}	4.3^{a}	9.4^{a}	51.5^{a}	76.7^{a}	33.4^{a}	13.6^{ab}
Mean	4.0	93 (9.6)	50.8	83.1	33.2	13.4	3.9	9.5	51.3	75.8	32.8	13.6
MSD	2.3*	0.5 (NS)	12.3 (NS)	6.3***	8.6(NS)	1.1***	2.15 (NS)	2.4 (NS)	7.7(NS)	13.6 (NS)	5.0 (NS)	1.1***
CV %	18.23	1.79	7.8	2.41	8.2	2.5	17.89	7.2	4.8	5.7	10.1	2.6

*(Significance at *p ≤0.05 and **p ≤0.001; NS- Non-significant; values in parentheses are square root transformed)*

The endophyte LAS 6 documented 3.0 days to seedling emergence, which was significant compared to the control (5.3 days). Percent seedling emergence was found to be non-significant, with PJ 9 recording a maximum value of 95% while control recorded a minimum value of 88%. Plant height was recorded at 45 and 90 days after sowing (DAS). Plant height at 45 DAS was found to be non-significant; however, the PJ-9 treatment recorded the highest value of 54.5 cm, while the control had 47.6 cm. Plant height at 90 DAS was found to be significant, with endophyte PJ 9 recording an increased value of 88.2 cm and control recording 81.9 cm. The physiological parameters, like SPAD readings, showed non-significant differences due to treatment and priming with bacterial endophytes. BA recorded the highest reading of 35.2, and LAS 6 had a minimum value of 30.8. The number of tillers per plant was found to be significant, where endophyte treatment with V4 J recorded a greater number of tillers (14.7), while control recorded a lower number of tillers per plant (12.5). Under drought conditions, most of the studied characters were found non-significant; however, the number of tillers per plant was found significant due to treatments. The endophyte, PJ 9, recorded a greater number of tillers per plant with a value of 14.4, while control recorded 12.1.

Effect of endophyte bio-priming on seed yield parameters in rice var. IR 64 under normal and drought conditions

The seed yield parameters were found to be statistically non-significant due to treatments. However, some of the endophytes recorded increased values compared to the control (Table 4.3.2). Under normal conditions, the bacterial endophyte BA recorded a greater number of panicles per plant (15.6), while the control recorded 11.3. The endophyte V4 J recorded highest panicle length (22.2 cm) and seed yield (3488.0 kg/ha). LAS-6 endophytes documented the maximum values for the characters such as branches per panicle (7.9), number of seeds per panicle (71.4), and harvest index (0.59).

Table 2: Effect of seed bio-priming with endophytes on seed yield parameter of rice var. IR 64 under normal and drought condition

Treatments	Normal condition						Drought condition					
	No. of panicles /plant	Panicle length (cm)	Branches / panicle	No. seeds/ panicles	Seed yield (kg/ha)	Harvest index	No. of panicles /plants	Panicle length (cm)	Branches /panicle	No. seeds/ panicles	Seed yield (Kg/ha)	Harvest index
Control	11.3^{a}	22.0^{a}	7.8^{a}	66.0^{a}	3243.6^{a}	0.54^{a}	12.2^{a}	21.5^{a}	7.4^{a}	52.4^{a}	1271.8^{a}	0.34^{b}
LAS-6	12.5^{a}	20.9^{a}	7.9^{a}	71.4^{a}	2824.0^{a}	0.59^{a}	12.7^{a}	21.9^{a}	7.6^{a}	69.8^{a}	2013.3^{a}	0.40^{ab}
PJ 9	13.8^{a}	21.5^{a}	7.8^{a}	69.9^{a}	3272.9^{a}	0.51^{a}	13.1^{a}	21.3^{a}	6.8^{a}	57.6^{a}	1660.6^{a}	0.36^{ab}
SF 5	13.6^{a}	21.6^{a}	7.6^{a}	69.6^{a}	3058.7^{a}	0.54^{a}	12.5^{a}	25.9^{a}	6.9^{a}	51.0^{a}	1659.4^{a}	0.42^{ab}
V4 J	13.2^{a}	22.2^{a}	7.6^{a}	67.4^{a}	3488.0^{a}	0.57^{a}	11.8^{a}	21.7^{a}	7.3^{a}	65.7^{a}	1653.1^{a}	0.39^{ab}
V6 E	14.8^{a}	21.1^{a}	7.8^{a}	71.4^{a}	2793.8^{a}	0.57^{a}	11.9^{a}	21.5^{a}	7.6^{a}	57.2^{a}	2229.4^{a}	0.47^{a}
BA	15.6^{a}	22.0^{a}	7.8^{a}	69.2^{a}	2756.4^{a}	0.50^{a}	12.6^{a}	21.1^{a}	7.2^{a}	47.7^{a}	1426.5^{a}	0.43^{ab}
Mean	13.5	21.6	7.8	69.3	3062.5	0.55	12.4	22.1	7.3	57.3	1702.0	0.43
MSD	6.1 (NS)	3.9 (NS)	0.9 (NS)	17.9(NS)	1278.4 (NS)	0.3 (NS)	7.6 (NS)	11.7 (NS)	1.6 (NS)	44.6 (NS)	636.2 (NS)	0.3** (NS)
CV %	15.1	2.7	3.7	8.8	28.7	15.3	19.5	7.0	7.0	24.9	25.7	24.0

*(Significance at p value **0.01; NS- Non-significant)*

Under drought conditions, PJ 9 endophyte treatment showed a greater number of panicles per plant (13.1), whereas panicle length was greater in the plants treated with SF 5 endophyte (25.9). Endophyte LAS 6 had recorded more branches per panicle (7.6) and the number of seeds per panicle (69.8). The treatment with V6 E recorded a maximum seed yield of 2229.4 kg/ha and a harvest index of 0.47.

Effect of endophyte bio-priming on growth and seed yield parameters under saline soil patch condition

All the characters studied were found to be statistically non-significant. However, the endophyte-treated plants recorded higher values compared to the control. The endophyte V4 J recorded maximum plant height (46.9 cm) at 45 days after transplanting, while PJ 9-treated plants had maximum plant height (84.5 cm) at 90 days after transplanting. The SPAD reading (42.0) was higher in the plants treated with LAS-6 endophytes. The endophyte SF 5 recorded the maximum values for number of tillers per plant (16.3) and number of branches per panicle (7.7). the seed yield characteristics, viz., number of panicles per plant (16.6), panicle length (19.7 cm), and seed yield (3090.5 kg/ha).

In the past, a limited number of studies have been conducted in field conditions utilizing endophytes. However, the research data provided by earlier researchers partially aligns with the data obtained in the current investigation. In a study conducted by Wijesooriya *et al.* (2016), it was demonstrated that the presence of *Acremonium* and *Arthrobotrys* sp., which are natural host endophytic fungi, led to enhanced plant growth in rice crops. This enhancement was observed in terms of increased height, tiller number, fresh weight, and dry weight. The study was conducted in both greenhouse and field environments. The combined fungal enrichment had a greater significant difference than the single inoculum and non-endophyte-treated paddy seedlings in terms of grain yield under field conditions, suggesting a significant role of endophytes in host plant growth promotion. Wijesooriya *et al.* (2016) Suggesting the use of consortiums would be better under field conditions. Treatment with indigenous rice root endophytic Azotobacter sp. strain Avi2 exhibited higher yield under field conditions (Banik *et al.*, 2019). Mastan *et al.* (2019) found that wheat bran-based fungal endophytes of *Coleus forskohlii* endophytic formulations significantly increased plant height, number of branches, root biomass, photosynthetic pigments, and forskolin content. The field data documented by Lally *et al.* (2017) demonstrated statistically significantly increased crop height, stem/leaf biomass, and pod biomass, particularly, in the consortium of Pseudomonas fluorescens strains. Although seed and oil yields were increased in the field in response to inoculation, they were found statistically non-significant under field conditions. Hajeevaka *et al.* (2018) studied root colonization of two bacterial endophytes, *Bacillus amyloliquefaciens* QST713 and *B. firmus* I-1582, and treated two corn and two soybean varieties grown in the field. They found that, two weeks after seeds were planted in the field, there were no significant differences in root colonization in either bacteria strain among varieties. Morsy *et al.* (2020), demonstrated that under greenhouse and field trials, *Ampelomyces* sp. and *Penicillium* sp. endophytes proved effective in conferring positive benefits to tomatoes placed under salt stress as well as under normal growing conditions by increasing yield.

Table 3: Effect of seed bio-priming with endophytes on growth and yield parameters in rice var. IR 64 under saline condition

Treatments	Plant height (cm)		SPAD reading	No. of Tillers/ Plant	No. of Panicles/ Plant	Panicle Length (cm)	No. of Branches/ Panicle	No. of Seeds /Panicle	Yield (Kg/ha	Harvest Index
	45 DAT	90 DAT								
Control	40.1[a]	77.6[a]	38.3[a]	14.2[a]	14.6[a]	18.7[a]	7.5[a]	46.7[a]	2710.6[a]	0.48[a]
LAS 6	42.4[a]	80.7[a]	42.0[a]	15.1[a]	15.6[a]	19.5[a]	7.5[a]	52.7[a]	3055.7[a]	0.48[a]
PJ 9	46.1[a]	84.5[a]	41.3[a]	15.9[a]	16.6[a]	19.7[a]	7.5[a]	49.1[a]	3090.5[a]	0.47[a]
SF 5	42.3[a]	79.7[a]	41.8[a]	16.3[a]	14.6[a]	18.7[a]	7.7[a]	45.0[a]	2755.2[a]	0.46[a]
V4 J	46.9[a]	80.6[a]	40.8[a]	15.5[a]	13.9[a]	18.8[a]	7.2[a]	47.2[a]	2355.9[a]	0.41[a]
V6 E	45.9[a]	80.9[a]	39.8[a]	14.9[a]	13.9[a]	19.0[a]	8.1[a]	49.5[a]	2800.9[a]	0.46[a]
BA	41.9[a]	81.3[a]	40.4[a]	15.1[a]	15.5[a]	19.2[a]	7.6[a]	43.2[a]	2955.0[a]	0.47[a]
Mean	43.7	80.7	40.6	15.3	15.0	19.1	7.6	47.6	2817.7	0.46
MSD	9.4 (NS)	8.1 (NS)	5.8 (NS)	2.7 (NS)	5.3 (NS)	1.4 (NS)	1.2 (NS)	17.7 (NS)	177.3 (NS)	0.3 (NS)
CV %	6.9	3.2	4.5	5.7	11.3	2.4	4.9	11.8	20.1	21.8

(NS- Non-significant)

The combination of different endophytic and rhizosphere bacteria has been tested to identify possible synergies of action and added benefits for the plant. Rice inoculated with a consortium of *Pseudomonas pseudoalcaligenes* and *Bacillus pumilus* exhibits a better response to salinity by accumulating higher levels of glycine-betaine-like quaternary ammonium compounds and biomass (Jha and Subramanian, 2011; Gupta *et al.*, 2022). The authors suggested that glycine- betaine may increase Na+ flux from the cytoplasm to the vacuole, maintain homeostasis, and stabilize the oxygen evolution from PSII. Similarly, the application of salt-tolerant Bacillus subtilis in combination with farmyard manure was shown to improve rice root and shoot biomass in saline soils (Bhambure *et al.*, 2018; Gupta *et al.*, 2020; Sannagoudar and Murthy 2018). Thus, the synergistic action of either different PGPB, or the mixture of PGPB with other organic amendments can overcome the adverse effects of salt stress on rice by regulating its growth and physiological performance. Hence, the use of these consortiums could also be considered for alleviating other abiotic stresses in rice.

The endophytes that were selected in the present study have been used for the first time under field conditions and individually. Most of the plant growth and seed yield characteristics were found to be non-significant, and a definite trend was not observed. However, endophyte bio-primed plants recorded increased values in plant growth and seed yield characteristics as compared to the control. The reasons for non-significant results under field conditions might be attributed to the heterogeneous nature of field soil conditions, where interactions of various soil microbiota, particularly soil and rhizosphere microbes, with treated endophytes play a major role in deciding the success of colonization of treated endophyte strains. Also, we need to standardize a load of endophytic inoculum separately for laboratory and field conditions, as we used a uniform inoculation load of 2 x 10^6 spores or mycelia per ml, which might also contribute to the results.

CONCLUSION

The present study data and previous researchers' data suggest that extensive and repetitive field experiments would be the need of the hour to validate and commercialize the use of endophytes to get positive and consistent results under field conditions. Further, a study on colonization efficiency and the development of endophytic strain consortium-based formulations will make this technology more successful.

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