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Seed Bio-priming with Fungal Endophytes for increased Seedling Performance in Rice var. IR 64

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ABSTRACT: Environmental stresses are limiting factors in optimal agricultural crop yield, and these stresses, especially drought and salinity, are likely to become more acute due to future climate change. Plant fungal Endophytes known to enhance early seedling vigour and growth particularly under stressful conditions. In order to enhance early seedling vigour a laboratory experiment was conducted using five fungal endophytes viz, LAS 6 (Chaetomium sp.), PJ 9 (Fusarium sp.), SF 5 (Fusarium sp.), V4 J (Botryosphariadothedia) and V6 E (Fusarium sp.) isolated from plant species grown in different habitat like drought, high temperature and saline region at Department of Seed Science and Technology, UAS, GKVK, Bengaluru. To assess the effect of endophytes, a seedling growth assay was conducted in rice var. IR 64 under normal condition (without stress), NaCl (170 mM) induced salinity stress and PEG-8000 (17 %) drought stress condition. The results showed that, the endophyte bio-priming had significantly increased early seedling growth and vigour. Under normal condition, the endophyte strain V4 J recorded significantly higher seedling length (42.2 cm), seedling dry weight (10.95 mg), and seedling vigour index I (3841) and II (1098) compared to control. Under NaCl induced salinity stress, the endophyte strain V6 E significantly increased the shoot length (13.1 cm) whereas, endophyte SF 5 had recorded significantly higher seedling length (24.0 cm), seedling vigour index I (2042) and II (807). Under PEG-8000 induced drought stress, V4 J strain had a maximum shoot length (14.6 cm), root length (23.7 cm) seedling length (38.3 cm) and seedling vigour index I (3213). The endophyte strain SF 5 has recorded a significantly higher seedling dry weight (8.79 mg) and seedling vigour index II (756) compared to control. The study concluded that, use of fungal endophytes can enhance early seedling growth and vigour under stressful conditions.

Keywords: Endophyte, Drought, Salinity, Seedling vigour, Bio-priming.

INTRODUCTION

The high-quality seeds have significant contribution in increasing the production potential of agricultural crops. Quality seeds with enhanced vigour contributes to nearly 30 % of total production potential of crops(Ellis, 2004). Early seedling vigour is most important attribute of quality seeds which can be enhanced through various seed-based treatment technologies. A wide range of seed-based techniques are now used in crop production to improve seedling vigour, establishment and growth under the changing environmental constraints.

Seed based treatment techniques may be differentiated into physical, physiological and biological seed enhancements. Under biological seed enhancements, various plant growth-promoting microbes have been used for many decades. Among various plant growthpromoting agents, plant endophytes are becoming more popular in agricultural research and have shown positive results in enhancing plant growth and development (Lin *et al.*, 2013). These endophytes can be used as seed bio-priming agents because of their ability to colonize diverse plant host systems through symbiotic nature. The bio-priming technique integrates both biological and physiological aspects to protect the seed and promote growth (Afzal *et al.*, 2016).

Endophytes includes bacteria, fungi, and unicellular eukaryotes are a class of plant-associated microorganisms that have shown potential in agriculture (Murphy *et al.*, 2013; Rodriguez *et al.*, 2009). They live at least part of their life cycle inter- or intra-cellularly inside the plants, usually without inducing any pathogenic symptoms. Bacterial and fungal endophytes have shown promise as beneficial crop inoculants, and many are known to enhance abiotic and biotic stress tolerance in plants. In the present study, an attempt has been made to evaluate the role of endophytes in enhancing early seedling growth and vigour through the seed biopriming technique in Rice (*Oryza sativa* L.) as the rice is the most important food crop grown around the world, 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 reliant on water availability. Extensive cultivation of rice under lowland conditions has posed secondary salinization problems and making soil saline. Due to the scarcity of water in agriculture, direct-seeded/aerobic rice cultivation is gaining momentum. In this context, the use of endophytes to make crop systems more tolerant specifically at early growth stage to abiotic stress has become one of the research interests in agricultural science in developing sustainable agricultural production technology. In this context, a study was conducted to assess the effect of endophyte bio-priming on early seedling growth and vigour in rice var. IR 64 under normal (without stress), drought and salinity stress condition.

MATERIAL AND METHODS

Endophyte isolates. Five fungal endophytes isolate were collected from the School of Ecology and Conservation, UAS, GKVK, Bengaluru and listed in Table 1.

Isolates	Location	Host	Fungal species
V4 J	Pokkali	Rice	Botryospharia dothedia
SF-5	Tamil Nadu	Suaeda filiformis	Fusarium sp.
LAS-6	Thar desert	Lasiurus scindicus	Chaetomium sp.
PJ-9	Karnataka	Prosopis juliflora	Fusarium sp.
V6 E	-	-	Fusarium sp.

Table 1: List of endophyte strains used in the study.

Seed material. Rice (*Oryza sativa* L.) var. IR 64 seeds were collected from Seed Unit, Zonal Agricultural Research Station (ZARS), Mandya.

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 haemo-cytometer under the light microscope. Further, the suspension concentration was adjusted to 2×10^6 spore/mycelia ml⁻¹ and used for bio-priming.

protocol. Seed bio-priming The mycelial suspension(2×10^6 spore/mycelia ml⁻¹) of the respective fungal isolate was used to bio-prime 48 h of pregerminated seeds and stirred occasionally for 3 h (Zhang et al., 2014). After 3 h of bio-priming, seeds were washed in sterile distilled water. One set of pregerminated seeds was soaked in sterile distilled water and used as a control treatment. Each treatment was maintained with 4 replications, each replication with 50 seedlings. The final germination percentage, root and shoot length, seedling length and seedling dry weight, seedling vigour index I and II were recorded at the end of the fourteenth day.

Induction of salt and drought stress. Salt stress was induced by using 170 mM (LC₅₀) NaCl salt solution by moistening paper towels and moisture was maintained for 14 days. The control and the paper towels were moistened regularly either with water or NaCl solution. Drought stress was induced by using 17 % (LC₅₀) PEG 8000 solution by moistening paper towels and moisture was maintained for 14 days. The control and the paper

towels were moistened regularly either with water or PEG-8000 solution.

Statistical design and analysis. Complete randomised design (CRD) and DMRT analysis were done using R - software.

RESULTS AND DISCUSSION

The experiments were conducted using five fungal endophytes under three different conditions *viz.*, normal condition (without stress), NaCl (170 mM) induced salinity stress, and PEG-8000 (17 %) induced drought stress. Seedling growth assay was conducted under laboratory condition to assess the effect of endophyte bio-priming in enhancing early seedling growth and vigour. Observations on final germination percentage, early seedling vigour traits like, shoot length, root length, total seedling length, seedling dry weight, and seedling vigour index (SVI) I and II were recorded.

Effect of endophyte bio-priming on early seedling growth and vigour under without stress condition. Under without stress condition, the endophyte strain SF 5 (Fusarium sp.) recorded the highest final germination of 92 % followed by endophyte strain PJ 9 and V4 J with 91 % germination. Control (without endophyte treatment) recorded the lowest final germination percentage of 85 % which was not statistically significant compared to better treatment. The endophyte strain V4 J (Botryosphariadothedia) recorded the highest seedling length of 42.2 cm which is statistically significant compared to the control which was recorded the lowest seedling length of 33.6 cm. the treatments V6 E, SF 5, and LAS 6 were recorded 40.0 cm, 38.7 cm and 38.4 cm seedling length and which were significantly on par with better treatment (Table 2).

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Treatments (Endophytes)	Final germination%	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Seedling Dry weight (mg)
Control	85 ^a	19.4 ^b	14.3 ^{bc}	33.6 ^b	10.95 ^b
LAS 6	89 ^a	20.9 ^{ab}	17.5 ^{abc}	38.4 ^{ab}	11.50 ^{ab}
PJ 9	91 ^a	20.6 ^{ab}	13.9°	34.5 ^b	12.03 ^a
SF 5	92 ^a	20.6 ^{ab}	18.1 ^a	38.7 ^{ab}	11.91 ^a
V4 J	91 ^a	24.7 ^a	17.5 ^{abc}	42.2 ^a	12.06 ^a
V6 E	88^{a}	22.2 ^{ab}	17.8 ^{ab}	40.0^{ab}	11.68 ^a
MSD	14.7 (NS)	4.1**	3.7**	7.6*	0.7***
CV %	5.7	6.5	7.7	6.8	2.1

Table 2: Effect of seed bio-priming with endophytes on seedling performance of rice var. IR 64 undernormal condition (without stress).

(Significance at p-value 0.0010.010.05; NS- Non-significant)

The results of present study were in agreement with previous studies where the increase in seedling growth was linked to the production of phytohormones by endophytes, namely gibberellic acids, auxins and cytokinins on rice growth. For instance, the *sphaerospermum* produces fungus Cladosporium gibberellins (GA₇ and GA₄), and the inoculation of this endophyte enhances rice biomass (Hamayun et al., 2009). Inoculation of plants with key growth regulators like indole acetic acid (IAA)-producing endophytic bacterium Burkholderia vietnamiensis improves rice growth and yield (Trân Van et al., 2000). IAA-producing endophytic fungal isolates from aromatic rice, positively regulate rice seed germination (Syamsia et al., 2015). Similarly, IAAproducing bacterial endophytes such as Micrococcus yunnanensis RWL-2, Micrococcus luteus RWL-3, Enterobacter soli RWL-4, Leclercia adecarboxvlata RWL-5, Pantoea dispersa RWL-6, and Staphylococcus epidermidis RWL-7, were reported to promote rice shoot and root elongation, biomass production and chlorophyll content (Shahzad et al., 2017a).

The endophyte strain V4 J recorded a maximum seedling dry weight of 12.06 mg which was statistically on par with other treatments Viz, PJ 9, SF 5, and V6 E with the value of 12.03 mg, 11.91 mg, and 11.68 mg respectively. While control recorded significantly lower seedling dry weight (10.95 mg) values compared to better treatment. The treatment V4 J had shown increased seedling vigour index I of 3841 which was significantly higher compared to control (2854) and on par with SF 5 (3559) and V6 E (3518). The endophytic strain V4 J had a maximum value for seedling vigour index II OF 1098 which was not significantly different from the control (930). Lalngaihawmi et al. (2018) reported similar results upon treatment with fungal endophyte resulted in increased per cent germination, shoot length and root length in rice compared to control. Rice seeds inoculated with fungal endophytes promoted the growth of rice seedlings in term of seed germination, plant height, root length and degree of root

colonization (Kundar et al., 2018). Zhi-lin et al. (2007) demonstrated similar results with significantly increased numbers of tillers, plant height, chlorophyll content, photosynthetic rate between endophyteinfected and endophyte-free plants, especially at the germination and seedling stages.

Effect of endophyte bio-priming on early seedling growth and vigour under NaCl (170 mM) induced salinity stress. To study the effect of seed bio-priming with endophytes under induced saline stress condition, the paper towels were moistened with 170 mM NaCl solution and bio-primed seeds were used to study germination and seedling growth parameters analysis (Table 3). There was no significant difference found in germination % between the treatments and control. However, the endophytic strains viz, V6 E, SF 5, PJ 9, and LAS 6 recorded the highest value of 85 % while, control recorded the lowest germination of 79 % (Table 1). The endophyte strain V6 E significantly increased the shoot length with the value of 13.1 cm compared to the control which had 7.9 cm. the treatment was on par with the endophyte SF 5 (12.8 cm) treatment. The endophytic strain SF 5 recorded the highest root length of 11.3 cm, which was statistically on par with the control (9.6 cm). Among the endophyte strains tested, SF 5 had recorded the highest seedling length of 24.0 cm and which was on par with the treatments V6 E (23.5 cm), LAS 6 (22.3 cm), and V4 J (21.7 cm) but, significantly higher than the control (17.5 cm).

The GA-producing endophytic Bacillus amyloliquefaciens RWL-1 enhances growth. photosynthesis and biomass of rice seedlings subjected to salt stress by increasing salicylic acid (SA) and essential amino acid levels resulted in improvement in rice growth (Shahzad et al., 2017b). these findings indicate that the reduction of endogenous stressresponsive hormones, such as the senescence promoting ABA (Song et al., 2016) and the growth-inhibiting JAs (Pérez-Salamó et al., 2019; Wang et al., 2020), represents a crucial mechanism employed by phytohormone-producing endophytes to mitigate different stress responses in rice.

Treatments	Final germination %	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Seedling DW (mg)
Control	79 ^a	7.9 ^d	9.6 ^{ab}	17.5 [°]	7.93 ^b
LAS 6	85 ^a	11.3 ^{bc}	11.1 ^a	22.3 ^{ab}	9.23ª
PJ 9	85 ^a	11.7 ^{abc}	9.1 ^b	20.8 ^b	9.20 ^a
SF 5	85 ^a	12.8 ^{ab}	11.3 ^a	24.0 ^a	9.50 ^a
V4 J	81 ^a	10.5 ^c	11.1 ^a	21.7 ^{ab}	9.68 ^a
V6 E	85 ^a	13.1 ^a	10.4 ^{ab}	23.5 ^{ab}	9.42 ^a
MSD	7.8 (NS)	1.54***	1.85**	3.00***	0.56***
CV %	2.98	4.6	6.0	4.7	2.12

 Table 3: Effect of seed bio-priming with endophytes on seedling performance of rice var. IR 64 under NaCl induced salinity stress (170 mM).

(Significance at p-value '***' 0.001 '**' 0.01; NS- Non-significant)

The Seedling dry weight was increased in seedlings treated with V4 J endophyte (9.68 mg) and which was on par with other treatments except for control (7.93 mg). The endophytic strain SF 5 treated seedlings showed increased seedling vigour index (SVI) I (2042) and seedling vigour index II (807) and it was significantly higher than the control which had SVI I of 1383 and SVI II of 626.

Fungal endophytes protect crops against abiotic stresses under laboratory conditions, as shown for salt (Baltruschat et al., 2008; Manasa et al., 2020). Megha et al., 2020, demonstrated that a salt-tolerant endophyte isolated from salt-adapted Pokkali rice, a Fusarium sp., colonizes the salt-sensitive rice variety IR-64, promotes its growth under salt stress and confers salinity stress tolerance its host. The GA-producing to amyloliquefaciens RWL-1 endophytic Bacillus enhances growth, photosynthesis and biomass of rice seedlings subjected to Cu stress and ameliorates the plant stress response by regulating Cu uptake, carbohydrate, and amino acid levels, and antioxidation (Shahzad *et al.*, 2019).

Endophyte and early seedling growth and vigour under PEG-8000 (17 %) induced drought stress. To study the effect of seed bio-priming with endophytes under drought stress condition, the paper towels were moistened with 17 % of PEG-8000 solution and bioprimed seeds were used to study germination and seedling growth parameters analysis (Table 4). The data on germination percentage was found non-significant due to treatments. However, LAS 6 endophyte recorded the highest germination percentage of 87 %, and the control recorded 81 %. The endophytic strain V4 J had a maximum shoot length of 14.6 cm which was on par with endophyte V6 E (13.6 cm), while the control recorded a significantly reduced shoot length of 11.9 cm.

Table 4: Effect of seed bio-priming with endophytes on seedling performance of rice var. IR 64 under PEG-
8000 induced drought stress (17 %).

Treatments	Final germination%	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Seedling Dry weight (mg)
Control	81 ^a	11.9 ^d	14.3 ^b	26.2°	7.47 ^d
LAS 6	87 ^a	12.1 ^{cd}	14.6 ^b	26.7 ^c	8.39 ^{bc}
PJ 9	86 ^a	13.3 ^{bc}	12.8 ^b	26.1°	8.33°
SF 5	86 ^a	13.3 ^{bc}	21.2 ^a	34.5 ^b	8.79 ^a
V4 J	84 ^a	14.6 ^a	23.7 ^a	38.3 ^a	8.67 ^{ab}
V6 E	85 ^a	13.6 ^{ab}	15.3 ^b	28.9 ^c	8.63 ^{abc}
MSD	0.72 (NS)	1.17***	2.68***	3.19***	0.30***
CV %	2.71	3.0	5.3	3.5	1.23

(Significant at p-value ***0.001; NS-Non-Significant)

The root length was significantly increased in the seedlings treated with endophyte V4 J (23.7 cm) which was on par with SF 5 (21.2 cm) but significantly higher than the control (14.3 cm). As for as seedling length is concerned, endophyte V4 J recorded a significantly higher seedling length of 38.3 cm compared to all other treatments and the control recorded a seedling length of 26.2 cm. Similar results were reported by earlier researchers are in agreement with our present study

where they showed that, reduction in stress-induced membrane damage in endophyte-inoculated rice, mirrored by lower malondialdehyde (MDA) content, has been reported (Li *et al.*, 2012; Kakar *et al.*, 2016; Jaemsaeng *et al.*, 2018; Qin *et al.*, 2019b; Shahzad *et al.*, 2019; Sun *et al.*, 2020; Tsai *et al.*, 2020). High ABA levels have been associated with reduced waterdeficit in endophyte- inoculated rice. ABA-producing Salicaceae endophytes reduce stomatal conductance, density and leaf water potential, enhancing water use efficiency (WUE) under drought conditions (Rho *et al.*, 2018).

The endophyte strain SF 5 has recorded a significantly higher seedling dry weight of 8.79 mg which was statistically on par with V4 J (8.67mg) and V6 E (8.63 mg) and the control recorded a lower seedling dry weight of 7.47 mg. Endophytic strain V4 J had recorded higher seedling vigour index I (3213) and it was on par with SF 5 (2967) while, the control had a significantly lower value of 2123. The seedling vigour index II was found significantly higher in seedlings treated with SF 5 endophyte (756) while control recorded lower SVI II of 605.

Comparable results were reported by earlier findings, where, fungal endophytes protect crops against abiotic stresses under laboratory conditions, as shown for heat and drought (Redman *et al.*, 2002; Bailey *et al.*, 2006; Hubbard *et al.*, 2014; Ali *et al.*, 2018) stresses.Similar results were reported using *P. indica*,

fungus has shown its multifarious functions in various fields like hardening of tissue culture plants, seedling germination, vegetative growth, early flowering, nutrient acquisition, increase yield, biotic stress tolerance and abiotic stress tolerance like drought, salinity, stress, heavy metal stress through various mechanisms (Singh et al., 2003; Sahay and Varma 1999; Waller et al., 2005; Sherameti et al., 2005; Yadav et al., 2010; Kumar et al., 2011; Jogawat et al., 2013; Das et al., 2012; Ansari et al., 2014; Rabiey et al., 2015; Ye et al., 2014; Hui et al., 2015; Sharma et al., 2015). Similarly, Sangamesh et al. (2018) evaluated thermo-tolerance of the isolates by culturing the fungi at 40 °C and 45 °C and showed that, LAS-6 (Chaetomium sp.) conferred high-temperature tolerance and other three OTUs, namely, LAS-4 (Aspergillus sp.), SAP-3 (Aspergillus sp.) and SAP-6 conferred drought tolerance in ricecultivar, IR-64, at the early seedling stage under drought stress.

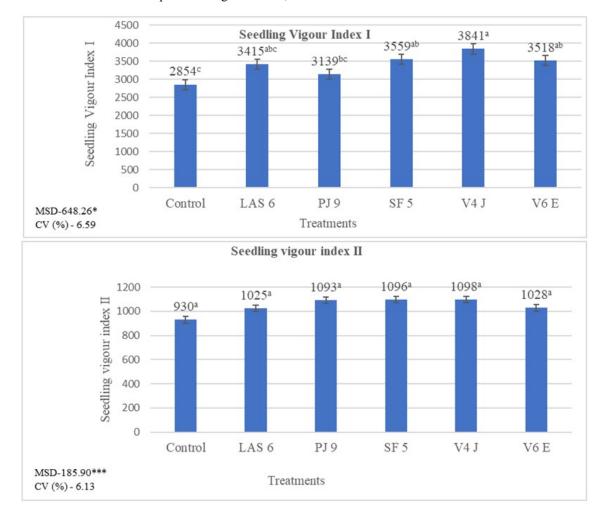


Fig. 1. Effect of seed bio-priming with endophytes on seedling vigour index of rice var. IR 64 under normal condition (without stress).

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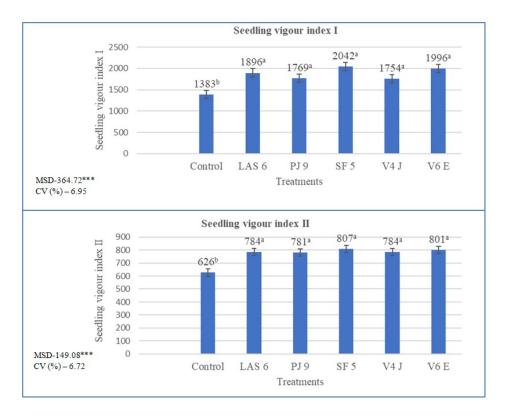


Fig. 2. Effect of seed bio-priming with endophytes on seedling vigour index of rice var. IR 64 seedlings under NaCl induced salinity stress (170 mM).

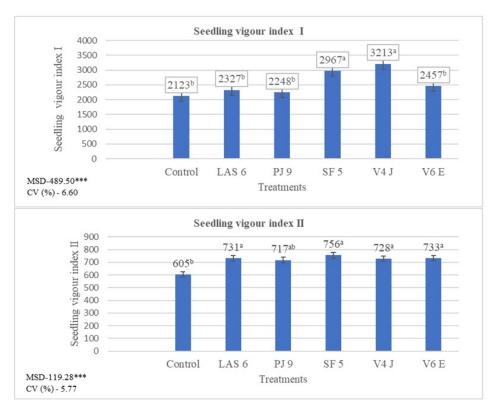


Fig. 3. Effect of seed bio-priming with endophytes on seedling performance of rice var. IR 64 under PEG-8000 (17%) induced drought stress.

CONCLUSION

The endophyte bio-priming can be a potent tool in enhancing early seedling growth and development under controlled conditions. The endophyte-enabled seed enrichment conferred tolerance to abiotic stress, particularly salinity and drought. The endophytes enhanced seedling and plant growth irrespective of stress and unstress plants however, the per se effect is more under stress conditions.

FUTURE SCOPE

Standardization of endophyte inoculum load or concentration for seed bio-priming in field conditions. Compatibility study of different endophyte strains both fungal with fungal and fungal with bacterial endophytes. Development of consortium of different endophytes which shows enhanced plant growth.

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