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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(12): 6069-6075

www.thepharmajournal.com
Received: 16-10-2022
Accepted: 18-11-2022

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Evaluation of seed priming with endophytes in imparting abiotic stress tolerance in rice var. IR 64

Shantharaja CS, Nethra N and Naveen K

Abstract
Endophytes are widely studied because of their positive association with plants in modulating growth and development. Four fungal endophytes were isolated from plants adopted to extreme environmental conditions like drought and salinity area. The study was conducted in pots under poly house. The experiments were set up in 3 conditions *viz.*, normal (without stress), salinity and drought stress conditions to evaluate effect of fungal endophyte bio-priming on morphological, physiological, and biochemical parameters in Rice Var. IR 64. SF5 had recorded increased plant height (37.8 and 33.8 cm) and root length (25.8 and 27.2 cm) under unstress and salinity stress (EC:4 dS/m), under drought stress (60% FC), V4J had shown increased plant height (36.2 cm) compared to control. The endophyte strain V4J had recorded lowest Malondialdehyde content of 0.129, 0.151 and 0.159 µg/g FW under unstress, salinity and drought stress respectively. Antioxidant enzyme (catalase, peroxidase, and superoxide dismutase) activity was higher in endophyte bio-primed plants under normal and stressful conditions in rice. The study concluded that, use of fungal endophytes can enhance early plant growth and antioxidant activity under stressful conditions.

Keywords: Endophytes, rice, abiotic stress, salinity, drought, and antioxidant enzymes

1. Introduction
Seed-based technologies have become a remedy to various agricultural problems. Seed-enabled technologies like novel seed enhancements and seed treatments which include priming, pelleting, coating, artificial seeds, etc., are applied in order to enhance seedling growth and better establishment of the crop. These techniques may be differentiated into physical physiological and biological seed enhancements.

Under biological seed enhancements, plant endophytes are becoming popular in agricultural research and have shown positive results in enhancing seed quality, plant growth and development, particularly early seedling vigour. These endophytes can be used as seed bio-priming agents because of their ability to colonize diverse plant host systems through symbiotic nature (Afzal *et al.*, 2016) [1]. These endophytes including bacteria, fungi, and unicellular eukaryotes are a class of plant-associated microorganisms that have shown potential in agriculture (Murphy *et al.*, 2014; Rodriguez *et al.*, 2009) [16, 34]. They live at least part of their life cycle inter- or intra-cellularly inside the plants, usually without inducing any pathogenic symptoms.

In recent times, the use of endophytic microbes has come up strongly in mitigating abiotic stress tolerance in crops. Salinity and drought stress are becoming more prominent and persistent throughout the world, posing great threats to sustainable agriculture production.

One of the most plausible explanations uncovered to date is that selected endophyte characteristics relieve reactive oxygen species (ROS) activity by enhancing anti-oxidative enzyme systems in host plants (Zhang *et al*., 2007; Rodriguez *et al*., 2008; Redman *et al*., 2011 and Bu *et al*., 2012) [44, 33, 32, 4]. ROS as a stress response agent which result in cell death in plants while anti-oxidative enzymes counteract to scavenge ROS. Another conceivable mechanism regards the ability to create phytohormones or to modulate phytohormone biosynthesis of host plants. Empirical data have supported the idea that auxin, gibberellic acid, abscisic acid, salicylic acid, and ethylene biosynthesis processes are likely related to the delay of stress responses in hosts (Cheng *et al*., 2012; Rasool *et al*., 2013; Khan *et al*., 2015 and Yaish *et al*., 2015) [5, 30, 16, 41].

To augment this concept of using endophytes in agriculture, the seed bio-priming method has shown its way in delivering beneficial microorganisms into the seed directly to take advantage of these microbes in improving seedling growth and development under stressful conditions.

In the present study, an attempt has been made to evaluate the role of endophytes in enhancing seedling growth, early plant establishment and plant growth through the seed bio-priming technique in rice (*Oryza sativa* L.).

2. Materials and Methods

The experiment was conducted at Department of Seed Science and Technology, University of Agricultural Sciences, Bangalore, Karnataka under three different conditions *viz*, normal (without stress), salinity stress (EC:4 dSm^{-1}) and drought stress (60% soil field capacity) in poly house. Four fungal endophytes were selected and each treatment was repeated 10 times. Initially, in each pot 5 pre-germinated seeds bio-primed with endophyte suspension (2 x 10^6 spore/mycelia ml^{-1}) were sown and after final emergence, one seedling was maintained in each pot. Various morphological, physiological, and biochemical parameters were recorded.

Treatment details

T_1-	Control	T_4-	V4J (*Botryospharia dothedia*)
T_2-	LAS6 (*Chaetomium* sp.)	T_5-	V6 E (*Fusarium* sp.)
T_3-	SF 5 (*Fusarium* sp.)		

2.1 Determination of field capacity (FC) of the soil

The field capacity was determined by the Gravimetric method as described by Earl (2003) [10]. Empty plastic plots were weighed using a weighing balance and the weight was used as an empty pot value (W). All the pots were filled with the potting mixture containing soil: sand: FYM (2:1:1) and their weight was used as a dry weight value (WD). These pots were saturated with water during evening hours to minimize the evaporation loss of water and the excess was allowed to drain. The weight of saturated soil was taken in the early morning and this weight was used as a wet weight value (WW). The field capacity was calculated as follows:

Weight of an empty plastic pot = W; Weight of Plastic pot + potting mixture= WD

Weight of Plastic pot + saturated soil = WW; Field capacity (100%) = (WW – W) – (WD - W). Similarly, 60% FC value was calculated.

2.2 Drought stress imposition by gravimetric approach.

The required level of field capacity was maintained by the gravimetric approach (Karaba *et al*., 2007) [14]. There were 5 treatments with 5 replications each. All pots were watered equally up to 100% field capacity until the 15^{th} day and later, field capacity was reduced in respective treatments to create drought stress up to 60 days. After 15 days after sowing, the treatments were watered to maintain 60% field capacity daily. All pots were weighed daily in the morning and stress was imposed on plants by withholding irrigation. During stress imposition, FC of 60% was maintained gravimetrically by checking the amount of water present in the soil medium compared to the maximum water it can hold by weighing a pot with soil daily and adding water to maintain the required soil field capacity level. The water status of the pot at 100% FC was maintained as control.

3. Mimicking of soil salinity in pots

The non-saline soils in pots were treated with a combination of sulfates and chloride salts to obtain desired soil EC, 4 dSm^{-1}. The protocol adapted to obtain desired soil EC, using a combination of salts is a standardized protocol developed by Central Soil Salinity Research Institute, (CSSRI, Karnal). This protocol mimics the natural soil saline conditions. The combination of salts required for obtaining the desired soil EC is listed (Table 1). Two kg soil-filled pots were saturated with salt water using a combination of salts to obtain the desired soil EC of 4 dSm^{-1} by checking the soil EC using the EC-TDS meter.

4. Observations recoded

4.1 Growth and physiological parameters

Plant height at 45 days after sowing, Number of leaves per plant, Root length, Root dry weight and SPAD chlorophyll meter reading (SCMR)- The relative leaf chlorophyll concentration of individual plants was measured using the hand-held dual-wavelength meter (SPAD 502, Chlorophyll meter, Minolta Camera Co., Ltd., Japan) -were recorded

4.2 Biochemical parameters

4.2.1 Malondialdehyde (MDA) estimation

One gram of tissue was homogenized with 5 ml of 5% aqueous TCA and 0.5 ml of 0.5% Methanolic BHT and heated for 30 mins in boiling water then the sample was centrifuged at 10000 g rpm for 10 mins then to 1ml of supernatant sample and 1 ml saturated TBA solution was added and kept in a boiling water bath for 30 mins. Centrifuged at 10000 g rpm for 10 mins and the absorbance was read at 532 nm (Draper and Hadley, 1990) [8]. Similarly, different concentration standard MDA solution was prepared using pure MDA and a series of standards were run in a spectrophotometer and a standard curve was prepared. The amount of MDA in the test sample was calculated from the standard curve

4.3 Antioxidant enzyme activity

4.3.1 Estimation of catalase (CAT) activity

The UV absorption of hydrogen peroxide was measured at 240 nm, by adding the substrate H_2O_2 to an enzyme extract. The enzyme breaks down the hydrogen peroxide and the resulting O_2 production produces bubbles in the reagent drop, indicating a positive test. Whose absorbance decreases when degraded by the enzyme catalase. From the decrease in absorbance, enzyme activity was calculated (Masia, 1998) [23].

4.3.2 Estimation of peroxidase (POD) activity

0.5 g of rice leaf tissue was taken and thoroughly ground with 5 ml of 50 µM citrate phosphate buffer (pH 6.4) with PVP and EDTA. The extract was centrifuged for 10 min. at 10000 rpm and the supernatant was used as an enzyme source for peroxidase. 3 ml of reaction mixture containing a buffer, Guaiacol and enzyme extract was taken. The increase in absorbance was measured at 450 nm up to 5 min for 1 min interval. The change in OD was obtained and peroxidase activity was calculated using the following formula and expressed as units/gm FW (Subhash, 1990) [36].

4.3.3 Estimation of superoxide dismutase (SOD) activity

Super oxides generated by the interaction of riboflavin and methionine in presence of light oxidize NBT compound into a violet colour. The superoxide dismutase enzyme reduces the oxidation of NBT by removing the superoxide formed during the reaction, thereby reducing the colour development. Therefore, the extent of reduction in colour development in the presence of the enzyme is proportional to enzyme activity (Du and Bramlage, 1994) [9].

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

6. Results and Discussion
6.1 Effect of endophyte bio-priming on growth and physiological parameters in rice var. IR 64 under normal condition
In this experiment rice plants were maintained in pots under ploy house conditions without any stress moisture is maintained at 100% soil field capacity. Observations on days to seedling emergence, % seedling emergence, plant height, number of leaves per plant, root length, root fresh weight, and dry weight, and SPAD readings were recorded. The results are tabulated in Table 1 and described in the following paras.

Table 1: Effect of seed bio-priming with endophytes on plant growth and physiological parameters of rice Var. IR 64 under normal condition

Treatments	Plant height (cm)	Number of leaves/ plants	Root length (cm)	Root DW (g)	SPAD Reading
Control	24.2[b]	13.8[c]	22.4[a]	1.03[b]	28.9[b]
LAS 6	28.6[b]	17.2[bc]	24.2[a]	1.18[b]	31.2[ab]
SF 5	37.8[a]	17.4[bc]	25.8[a]	2.06[a]	36.0[ab]
V4 J	35.8[a]	21.0[ab]	25.8[a]	1.95[a]	35.6[ab]
V6 E	35.8[a]	22.8[a]	24.6[a]	1.46[b]	36.4[a]
Mean	32.4	18.4	24.6	1.53	33.6
MSD	6.6***	4.9***	7.0 (NS)	0.5***	7.5**
CV%	8.4	11.0	11.7	12.3	9.1

(Significance at p - value '***0.001, **0.01, *0.05; NS: Non-significant)

Plant height showed significant differences between treatments and control in which treatment SF 5 recorded highest plant height of 37.8 cm whereas, the control recorded lowest plant height of 24.2 cm. in case of number of leaves per plant, the endophyte strain V6 E had recorded highest value (22.8) whereas, the control recorded decreased value (13.8). Root dry weight was maximum in plants treated with SF 5 (2.06 g) followed by V4 J (1.95 g) which was statistically on par. Whereas, the control plants recorded lowest value of 1.03 g. The SPAD readings were found significant due to treatments, and endophytic strain V6 E recorded increased SPAD reading of 36.38 whereas, control recorded 28.92.

6.2 Effect of endophyte bio-priming on growth and physiological parameters in rice var. IR 64 under saline condition
In this experiment rice plants were maintained in pots under ploy house conditions with salinity stress (EC: 4 dS/m). The results showed that, endophyte strain V4 J had recorded a maximum plant height of 33.8 cm whereas, the control had lowest value of 22.8 cm.

Table 2: Effect of seed bio-priming with endophytes on plant growth and physiological parameters in rice var. IR 64 under salinity stress

Treatments	Plant Height (cm)	No. of leaves/ plant	Root Length (cm)	Root DW (G)	SPAD Reading
Control	22.8[b]	9.6[a]	20.0[c]	1.02[c]	26.2[a]
LAS 6	28.4[ab]	10.2[a]	23.4[ab]	1.56[bc]	27.6[ab]
SF 5	33.4[a]	10.6[a]	25.0[ab]	1.43[ab]	31.8[ab]
V4 J	33.8[a]	12.8[a]	27.2[a]	1.69[ab]	32.7[bc]
V6 E	32.8[a]	11.6[a]	27.0[a]	1.95[a]	33.7[c]
Mean	30.1	10.96	24.2	1.53	30.2
MSD	9.2***	4.0 (NS)	4.7***	0.04***	6.4***
CV%	10.7	15.2	6.8	9.1	7.4

(Significance at p - value '***0.001, *0.05; NS: Non-significant)

Root length was found statistically significant due to the treatment effect where, V4 J recorded a maximum root length of 27.2 followed by V6 E (27.0 cm). Whereas, control recorded root length of 20.0 cm. Root dry weight was more in the plants treated with V6 E (1.95 g) endophyte whereas, the control had 1.02 g. In physiological parameter, SPAD reading showed a significant difference due to endophyte priming where, V6 E recorded maximum reading of 33.7 followed by V4 J (32.7). Whereas, the control recorded lowest value of 26.2.

6.3 Effect of endophyte bio-priming on growth and physiological parameters in rice var. IR 64 under drought condition
In this experiment rice plants were maintained in pots under ploy house conditions under drought stress by maintaining soil moisture at 60% soil field capacity (FC). Endophyte inoculated plants showed elevated plant height compared to control where, V4 J treatment had a maximum plant height of 36.2 cm followed by V6 E (35.0 cm) and SF 5 (34.2), whereas control recorded a decreased plant height (26.4 cm). Further, the treatment V6 E had recorded a greater number of leaves with a value of 11.6 which was significantly different compared to the control (7.8). Treatment SF 5 recorded a maximum root length value of 27.4 cm whereas control had 21.0 cm. In case of root dry weight, the treatment V6 E had recorded the highest value of 2.64 g, whereas the control had 1.22 g.

The data on SPAD reading showed significant differences due to endophyte treatment compared to control. The treatment V6 E had shown increased SPAD reading of 35.64 and which was on par with other endophyte treatments. Whereas, control recorded the lowest value of 27.3.

Table 3: Effect of seed bio-priming with endophytes on plant growth and physiological parameters of rice var. IR 64 under drought stress

Treatments	Plant height (cm)	Number of leaves/ plants	Root length (cm)	Root DW (G)	SPAD reading
Control	26.4[c]	7.8[b]	21.0[b]	1.22[b]	27.4[b]
LAS 6	30.2[bc]	8.8[ab]	22.8[b]	1.18[a]	33.3[ab]
SF 5	34.2[ab]	11.2[a]	27.4[a]	1.99[a]	34.9[a]
V4 J	36.2[a]	11.4[a]	23.8[ab]	2.49[a]	34.7[a]
V6 E	35.0[ab]	11.6[a]	24.2[ab]	2.64[a]	35.6[a]
Mean	32.4	10.2	23.8	1.9	33.2
MSD	5.0***	3.7**	4.5***	0.7**	7.2*
CV%	6.4	13.7	7.8	14.6	8.9

(Significance at p - value '***0.001, **0.01, *0.05; NS: Non-significant)

In the present study, four fungal endophytes were treated with rice pre-germinated seeds and grown in pots under polyhouse with drought and salinity stress along with normal growth condition. The results of the present study show agreement with previous studies conducted in different crop host systems.

Fungal endophytes conferred salt and drought tolerance to two commercial rice varieties that were not adapted to these stresses. Moreover, these endophytes reduced water consumption by 20%-30% while increasing the growth rate, reproductive yield, and biomass of greenhouse-grown plants (Redman *et al.*, 2011) [32]. A significantly positive/neutral effect of fungal endophyte was recorded under water-stressed situations. In general, the presence of fungal endophytes increased the plant's total biomass, chlorophyll content, and stomatal conductance irrespective of water availability (Dastogeer, 2018) [6].

Endophytes from saline and drought habitats can impart salt stress tolerance to paddy cultivars in a habitat-specific manner through symbiotic association. In previous studies treatment of *Piriformospora indica* with barley, *Arabidopsis*, and tomato conferred salinity stress (Baltruschat *et al.*, 2008) [3]. The symbiotically induced growth response was observed in tomato, *and* cucumber plants when treated with *Trichoderma harzianum, Penicillium janthinellum, Exophiala* sp. (Mastouri *et al.*, 2010; Khan and khan 2013; Khan *et al.*, 2012) [24, 17-18].

In paddy seedlings enhanced growth was observed upon treatment with *Fusrium culmorum*, *Piriformospora indica*, *Trichoderma harzianum* under salinity stress (Redman *et al.*, 2011; Jogwat *et al.*, 2013; Rawat *et al.*, 2012; Yasmeen and Siddiqui, 2017 and Yasmeen and Siddiqui, 2018) [32, 12, 31, 42-43]. The increase in SPAD values under stress condition upon treatment with endophytes observed in this study agree with previous reports (Redman *et al.*, 2011; Khan *et al.*, 2012; Waqas *et al.*, 2015; Jogawat *et al.*, 2013; Prajjal, 2016 and Lubna *et al.*, 2018) [32, 18, 40, 12, 28, 21].

Similar results were reported earlier with the use of dark septate endophytic fungi from wild rice which promoted rice growth and biomass production under water deficit conditions and reduced the effects of water deficit-induced oxidative stress in rice by scavenging ROS (Dos Santos *et al.* 2017) [7].

Beneficial fungal and bacterial endophytes have the potential to promote the growth and development of numerous plant species via a high degree of symbiotic association via colonization in endophytic nature (Rodriguez *et al.*, 2009; Zuccaro *et al.*, 2011) [34, 45]. Under adverse environmental conditions such as salinity, and drought, plants tend to develop a symbiotic relationship with beneficial microorganisms (Lum and Hirsch, 2002) [22].

4. Effect of endophyte bio priming on MDA content and anti-oxidant enzyme activity in rice var. IR 64 under normal, drought, and saline condition.

Endophytes are known to act positively on biochemical pathways which are known to enhance tolerance levels under stressful conditions. Among different biochemical parameters, the present study concentrated on the MDA content, and antioxidant enzyme activities of catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) were assayed in endophyte-treated and untreated plants grown under given unstress, salt stress (4 dSm^{-1}) and drought stress (60% soil FC).

The product of lipid peroxidation reaction that occurs due to stress conditions is MDA. This bio-molecule content is negatively correlated with the performance of the plant system, Lower the content of MDA higher the tolerance level of plants to stressful conditions.

In the present study same trend was observed where endophyte-treated plants recorded decreased quantity of MDA content irrespective of the condition i.e., unstressed, drought, and saline stress. The plants treated with endophyte strain V4 J produced a lower quantity of MDA content of 0.129 µg/g FW, 0.151 µg/g FW, and 0.159 µg/g FW under unstressed, drought, and saline conditions respectively. Whereas, untreated plants recorded increased proline content of 0.273 µg/g FW 0.934 µg/g FW, and 0.329 µg/g FW under unstressed, drought, and saline conditions respectively (Table 4.). Similar results have been reported earlier with a reduction in stress-induced membrane damage in endophyte-inoculated rice, mirrored by lower malondialdehyde (MDA) content (Li *et al.* 2012; Kakar *et al.* 2016; Jaemsaeng *et al.* 2018; Qin *et al*. 2019; Sun *et al.* 2020; Tsai *et al.* 2020) [19, 13, 11, 29, 37, 38].

In the case of the activity of antioxidant enzymes, endophyte-treated plants showed a significant increase in the level of CAT, POD, and SOD activity compared to untreated control plants under unstressed and stressed conditions.

Under the unstressed condition, SF 5 recorded increased CAT enzyme activity with the value of 886.25 U/g FW whereas, the control recorded a lower activity of CAT enzyme of 678.92 U/g FW. The POD activity was more in the plants treated with V4 J (507.0 U/g FW) which is significantly different compared to the control (291.20 U/g FW). SOD activity was found significantly higher in plants treated with V6 E endophyte with 62.12 U/g FW whereas, the control recorded 36.25 U/g FW.

The endophyte-treated plants under drought stress conditions showed increased enzyme activity compared to the control. CAT activity was elevated in the plants treated with V6 E with 1140.31 U/g FW whereas the control had 952.65 U/g FW. The plants treated with LAS 6 Endophyte strain recorded

678.11 U/g FW of peroxidase activity and control plants had 390.50 U/g FW. The SOD activity was increased in the plants treated with V6 E endophyte with 82.10 U/g FW, whereas control plants had 57.80 U/g FW.

Under saline stress conditions, endophyte LAS 6 treated plants recorded significantly higher CAT activity with 1148.22 U/g FW compared to the control, which had recorded 764.77 U/g FW. The POD activity was more in the plants treated with endophyte V6 E where it recorded 852.17 U/g FW and the control recorded 424.57 U/g FW. The SOD activity was elevated in the plants treated with the endophyte SF 5 with a value of 90.27 U/g FW and which was significantly higher compared to untreated plants (65.87 U/g FW).

Liang Li *et al.* (2017) [20] observed reduced malondialdehyde (MDA) activity, Na+ content, and relative electrolyte conductivity (REC) in *P. indica* colonized plants. Especially under stressful conditions. Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) were reduced in endophytic plants under stress as compared with non-endophytic counterparts. Categorical analysis revealed that accumulation in plant biomass is influenced by factors such as host and fungi identity, the magnitude of which is greater under stress than non-stress conditions (Dastogeer, 2018) [6]. Incidentally, a 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.*, 2019; Shahzad *et al.*, 2019; Sun *et al.*, 2020; Tsai *et al.*, 2020) [19, 13, 11, 29, 35, 37, 38].

The endophytic fungi from upland rice mediate rice drought tolerance by detoxifying ROS through increased superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities in the inoculated seedlings (Pang *et al.*, 2020). In another case, rice plants treated with a consortium of two rhizobacteria (*Bacillus amyloliquefaciens* and *Brevibacillus laterosporus*)

Similar results were reported as the endophytic fungi from upland rice mediate rice drought tolerance by detoxifying ROS through increased superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities in the inoculated seedlings (Pang *et al.*, 2020) [27]. The increase in antioxidant enzyme activity due to infection with endophytes used under different stress conditions has in agreement with previous studies in different crop systems. Waller *et al.* (2005) [39] and Baltruschat *et al.* (2008) [3], showed inoculation of *P. indica* to barley increases glutathione-ascorbate cycle activity and enhanced antioxidant capacity thus inhibiting the NaCl-induced lipid peroxidation, metabolic heat efflux, and fatty acid desaturation in leaves of the salt-sensitive barley under salt stress. Khalid *et al.* (2018) [15], demonstrated *P. indica* co-cultivation significantly increased antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT).

Baltruschat *et al.* (2008) [3], studied the biochemical mechanisms underlying *Piriformospora indica* mediated salt tolerance in barley and endophyte significantly elevated the amount of ascorbic acid, and increased the activities of antioxidant enzymes. The findings suggest that antioxidants might play a role in both inherited and endophyte-mediated plant tolerance to salinity. Similar results have been obtained in various crop plants under salt stress upon endophytic fungal treatments (Waqas *et al.*, 2015; Baltruschat *et al.*, 2008; Khalid *et al.*, 2018).

Table 4: Effect of seed bio-priming with endophytes on MDA and antioxidant enzyme activity in leaves of rice var. IR 64 under normal, drought, and saline conditions

Treatment	Normal				Drought (60% FC)				Saline (EC: 4dSm-1)			
	MDA (µg/g FW)	Catalase (U/g FW)	Peroxidase (U/g FW)	SOD (U/g FW)	MDA (µg/g FW)	Catalase (U/g FW)	Peroxidase (U/g FW)	SOD (U/g FW)	MDA (µg/g FW)	Catalase (U/g FW)	Peroxidase (U/g FW)	SOD (U/g FW)
Control	0.273a	678.9d	291.2e	36.3d	0.934a	952.7a	390.5e	57.8c	0.329a	764.8d	424.8d	65.9b
LAS 6	0.222a	875.2b	382.7d	46.3c	0.344b	1048.5a	678.1a	79.4a	0.238b	1148.2a	640.4b	86.1a
SF 5	0.146b	886.3a	411.9c	58.6ab	0.218c	1090.6a	617.4b	69.6b	0.163b	983.4bc	564.4c	90.3a
V4 J	0.129b	670.8e	507.0a	55.0b	0.151c	1061.7a	539.7d	70.5b	0.159b	910.6cd	580.9c	75.9ab
V6 E	0.148b	843.4c	470.9b	62.1a	0.188c	1140.3a	606.0c	82.1a	0.229b	1114.3ab	852.2a	79.5ab
Mean	0.18	90.9	412.7	51.7	0.367	1058.7	566.3	71.9	0.22	984.3	612.5	80.0
MSD	0.07**	6.4**	9.5**	7.1**	0.12**	209.0 (NS)	11.2**	3.5**	0.08**	151.6**	20.6**	22.3**
CV%	13.4	0.3	0.8	4.8	11.8	6.9	0.7	1.7	12.4	5.3	1.2	9.7

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

Conclusion

The endophyte-enabled seed enrichment conferred tolerance to abiotic stress, particularly salinity and drought. The endophytes strain V4 J, SF 5 and V6 E enhanced seedling and plant growth under both stress and unstress conditions. The increased tolerance to stress conditions was attributed to the elevated activity of antioxidant enzymes in plants treated with V6 E, SF 5, and LAS 6 endophyte strains. The endophyte V4 J conferred stress tolerance by producing a reduced quantity of malondialdehyde (MDA). The endophyte V4 J (*Botryospharia dothedia)*, SF 5 (*Fuasarium* sp.), and V6 E (*Fusarium* sp.) in rice can be used as potential bio-priming agents to enhance early seedling growth and plant performance under both stress (drought and salinity) and unstress condition.

Acknowledgment

The authors gratefully acknowledge ICAR-CAAST, Project (activity 1c), UAS, GKVK, Bengaluru, and ICAR-CAZRI, Jodhpur for their financial support and Prof. R. Uma Shaanker for providing endophytes cultures.

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