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Research article

Modulation of plant growth and antioxidative defense system through endophyte biopriming in cotton (*Gossypium* spp.) and non-host crops

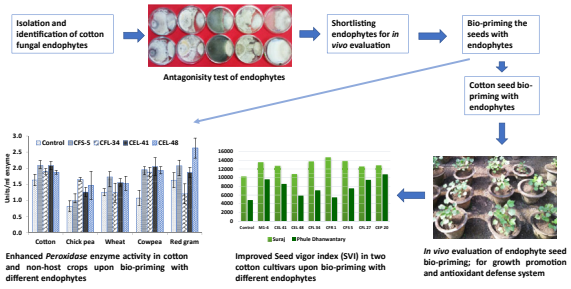
Pooja Verma [a,1], Neelakanth S. Hiremani [a,*,1], Shailesh P. Gawande [a,1], Satish K. Sain [b], Dipak T. Nagrale [a], Nandini G. Narkhedkar [a], Y.G. Prasad [a]

[a] ICAR-Central Institute for Cotton Research, Nagpur, 440010, Maharashtra, India
[b] ICAR-Central Institute for Cotton Research, Regional Station, Sirsa, Haryana, India

HIGHLIGHTS

- Fungal endophytes of cotton were antagonistic to *Corynespora cassiicola* and *Fusarium solani* inhibiting up to 66% of growth.
- Seed biopriming of two cotton cultivars Suraj and Phule Dhanwantary with endophytes enhanced seed germination and seed vigor.
- Endophytes also benefited non-host crops like wheat, sorghum, chick pea and cow pea wherein seed germination was enhanced
- Endophyte biopriming had positive effect on plant growth promotion and antioxidative defense system in all the treated crops.
- Increase in total soluble protein, total sugar, *catalase* and *peroxidase* activity was visible in endophyte treated plants.

GRAPHICAL ABSTRACT



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ABSTRACT

Seed biopriming is very promising in improving seed health by mitigating various biotic and abiotic stresses. In this study, the effect of biopriming with cotton endophytes on seed germination and other growth parameters in host and non-host crops like wheat, sorghum, cowpea and chick pea was examined. The endophytes were antagonistic to cotton pathogens *Corynespora cassiicola* and *Fusarium solani* under *in vitro*. Among the eight endophytes, CFR-1 and CEL-48 were highly efficient with inhibition rates of 66.16% and 64.24% respectively against *C. cassiicola*, whereas CFL-34 was efficient against *F. solani* with more than 50% inhibition. Seed biopriming enhanced seed germination in cotton and non-host crops whereas seed vigor index was highest in bioprimed cotton. Moreover, growth promotion parameters were also enhanced upon endophyte biopriming. Total sugar content ranged from 5.46 to 7.54 mg/g F.W in cotton and highest was found in CFL-34 treated wheat (8.64 mg/g FW). There was an increase of 10–30% soluble protein in bioprimed cotton over control. Interestingly, the antioxidant potential in all the bio-primed crops was improved with increased catalase and peroxidase activity. Specific activity of catalase ranged from 0.42 to 1.90 μmol/min/mg protein in cotton, while highest activity

* Corresponding author.
E-mail address: nhneelmani@gmail.com (N.S. Hiremani).
[1] These authors have equal contribution.

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was reported in CEL-48 primed wheat. The findings of this investigation emphasizes seed biopriming with endophytes for sustainable plant health management.

1. Introduction

Quality seed is the most critical input in agriculture and the increased global demand has necessitated to employ new strategies to cater to the needs of sustainable agriculture. The seed quality improvement is a major challenge as it involves improving the seed germination, seed vigor, seed viability and seed health. Seed priming with beneficial microorganisms (biopriming) has been found to be very promising (McDonald 2000; Van Hulten et al., 2006; Kumar et al., 2016) to improve seed health and mitigate the adverse impact of various biotic and abiotic stresses. Biopriming, with beneficial fungi, bacteria or actinomycetes, is a viable and promising approach to enhance seed uniformity and plant establishment (Kumar et al., 2020). The microbiome of plants is an active area of research interest owing to its enormous benefits to host plants. Endophytes occur ubiquitously in cultivated crops as well as in non-cultivated plants. They inhabit the healthy plant tissues, at least for part of their life cycle, but do not harm the host plants. The focus on endophytic microbes is expanding nowadays due to their edge over the rhizospheric microbes, because, biopriming with endophytes at favorable temperatures allows sufficient time for them to enter and colonize the seeds (Mahmood and Kataoka 2019). Moreover, several fungal and bacterial strains have been applied as plant or seed inoculants, in various crops, that develop a symbiotic relationship with their host and thereby benefit them by enhancing the growth and yield. Clay (1987) investigated the effect of endophyte treatment on seed biology and seedling vigor of tall fescue and perennial rye grass. It was found that the filled seeds produced by endophyte infected tall fescue were two times high as compared to uninfected plants, while there was no significant change in case of perennial rye grass. Both, infected tall fescue and perennial rye grass, produced significantly more biomass and tillers after 10–14 weeks of growth as compared to uninfected plants. The classic example of root endophytic fungus *Piriformospora indica*, to enhance disease resistance, salt stress tolerance and increase in grain productivity of barley is very well documented (Waller et al., 2005). Similarly, the antagonistic potential of fungal endophytes has been proven in crops like maize, wheat and recently in cotton (Wicklow et al., 2005; Jaber 2018; Hiremani et al., 2020). Endophyte mediated induction of disease resistance has been observed to be systemic in nature. Swain et al. (2021) reported that seed biopriming with *Trichoderma* strains reduced mean germination time, enhanced the seedling vigor and total chlorophyll content. Further, they also found higher quantities of growth promoting indole acetic acid, prussic acid and higher expression of defense enzymes like peroxidase, catalase, polyphenol oxidase and superoxide dismutase. In another study, seed biopriming of soybean with *Trichoderma harzianum* strain BS1 showed positive correlation with soybean growth factors resulting in enhanced shoot and root length, seedling dry weight and total chlorophyll content (Entesari et al., 2013). We hypothesize that, endophytes equipped with growth promotion and antagonistic property, *i.e.* capable of inhibiting plant pathogens, would be an added advantage and most suitable candidates for seed biopriming because they fulfil both the production and protection strategies. Thus, an experiment was carried out with the objective to investigate the effect of biopriming with promising fungal endophytes from cotton (*Gossypium* spp.) on germination parameters and antioxidative defense system in host as well as non-host crops.

2. Material and methods

2.1. Collection of samples, isolation and identification of endophytic fungi

Leaf samples of *Gossypium hirsutum* and *G. arboreum* were collected from cotton fields. The samples were brought to the laboratory and thoroughly washed under running tap water to remove debris, if any. Isolation of endophytes was done as per the protocol (Li et al., 2014) with slight modification as described in our previous work (Hiremani et al., 2020). In brief, thoroughly washed leaves were surface sterilized with ethanol (70%) and sections were made with a sterile blade. These leaf sections were treated with Sodium hypochlorite for 1 min and washed thrice with sterile water. Finally, dipped in ethanol (70%) for 20 s and blotted on a sterile filter paper. These leaf sections were put on PDA medium and incubated at 28 ± 1°C for 10 days. Pure cultures of the endophytic fungi were used for identification (Figure 1). Based on the conidial characters preliminary identification was done. Besides, internal transcribed spacer (ITS) region is widely used to identify the fungal species through ITS sequences because of its small size and highly conserved flanking sequences. Therefore, universal ITS 1 and ITS 4 markers (White et al., 1990) were used for confirmation of the species.

2.2. Antagonistic potential of endophytes

Antagonistic activity of the endophytic fungi was tested by dual culture technique (Bell et al., 1982). Eight fungal endophytes were evaluated *in vitro* against two important pathogens, *Corynespora cassiicola* and *Fusarium solani*, isolated from diseased cotton plants (Figure 1). Pathogenicity of both the pathogens was performed on cotton (cv. Suraj). For confrontation or dual culture assay, a 5 mm disc each of 7 days old fungal endophyte and pathogen were placed on PDA medium in the same Petri dish at the opposite end. Plates inoculated with only the pathogen were served as control and the experiment was replicated thrice. The triplicate plates were then incubated at 28±1°C in a BOD incubator (Osworld, India). Observations on colony growth were made and colony inhibition percentage (Vinale et al., 2008) was calculated by the following Eq. (1).

$$\text{Colony Inhibition percentage} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100 \quad (1)$$

2.3. Effect of endophyte biopriming on germination percentage and seed vigor index in cotton

The effect of fungal endophytes on seed germination and vigor index of cotton was tested through seed priming. The conidia of eight fungal endophytes were harvested (10 dpi) separately under sterile conditions and the method described by Jaber and Enkerli (2016) was followed for preparation of the spore suspension. In brief, conidia were harvested by flooding the culture plate with Tween 80 (0.01%) and gently stirring with a glass rod. The suspension was filtered and pure conidial suspension was used for seed treatment. Later, the seeds of two cotton cultivars *viz.*, Suraj (*G. hirsutum*) and Phule Dhanwantary (*G. arboreum*) were treated with conidial suspension of each fungal endophyte (1×10^7 spores/ml) by seed soaking for 4h and then ten seeds for each endophyte were sown in a paper towel with three replications and kept in dark at 28±1 °C for designated duration. Seeds soaked in sterile distilled water for 4 h served as control. Inoculated and control seeds were then used in germination test and observations on root length and shoot length were recorded for calculating the seed vigor index (SVI, expressed in whole number) (Abdul-Baki and Anderson 1973)) by using Eq. (2) as follows,

$$\text{Seed Vigor Index (SVI)} = \text{Germination percentage (\%)} \times \text{Total seedling length (mm)} \quad (2)$$

2.4. Effect of seed biopriming on germination of different non-host crops

In this study, we bioprimed the seeds of different non-host crops *viz.*, wheat, sorghum, chick pea and cowpea with fungal endophytes' spore

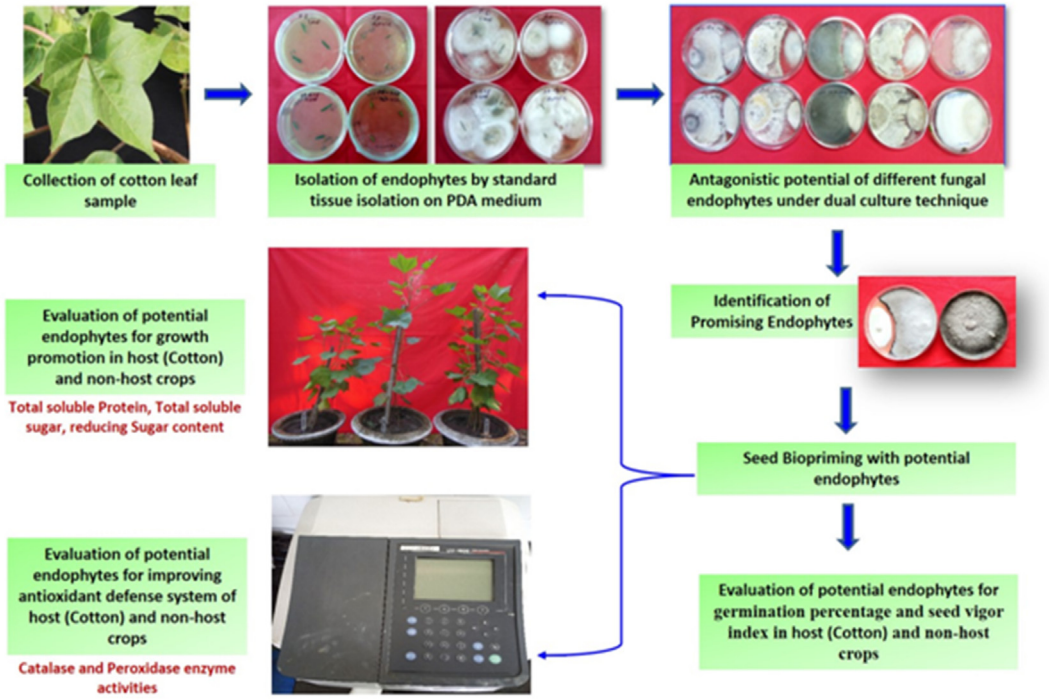

Figure 1. Flow diagram of different steps involved in Materials and Methods.

suspension (1×10^7 spores/ml) for 4 h. In all the cases, spore suspension (1×10^7 spores/ml) was prepared from 10 days old culture as mentioned above. Seeds not primed with fungal endophytes were served as control. Bio-primed seeds were then sown in disinfected pots (5 seeds/pot) filled with sterile soil and replicated thrice. Germination count of each non-host crop was taken at 10 DAS and germination percentage was calculated.

2.5. Effect of biopriming on plant growth promotion and antioxidative system

2.5.1. Estimation of protein content

The protein accumulation was estimated using the protocol of Bradford (1976). In brief, 500 mg of leaf tissue was crushed using 1 ml phosphate buffer (pH 7) and then centrifuged @ 12000 rpm for 10 min at 4°C. For protein estimation, BSA standards of different known concentrations were prepared. Blank was prepared by adding 1 ml distilled water to the 2 ml Bradford reagent. Likewise, samples were prepared by adding 10μl protein extract to the mix of 990μl distilled water and 2 ml Bradford's reagent and were incubated at room temperature for 10 min. Absorbance was taken at 595 nm wavelength. The protein concentration was calculated from standard graph.

2.5.2. Total soluble sugar

The amount of total soluble sugars was estimated using anthrone method (Thimmaiah 2012). Leaf tissue (0.1g) was boiled in 5 ml 2.5 N HCl for 3h and then neutralized with solid sodium carbonate. The final volume was made up to 100 ml and aliquots of 0.5 and 1ml were used for analysis. The intensity of color formed after adding anthrone reagent was read at 620 nm and concentration of total soluble sugars was calculated using standard curve of glucose.

2.5.3. Estimation of reducing sugar

Reducing sugars from leaves of host and non-host crops (as above) were estimated using Nelson-Somogyi's method (Nelson 1944). Samples (0.1 g) of control and bio-primed plants were homogenized in 80% ethanol. The extract was centrifuged at 10,000 g for 15 min at room temperature; further supernatant was evaporated by keeping the homogenates in water bath at 80°C. Reducing sugars were estimated by using alkaline copper tartrate and arsenomolybdate reagent colorimetrically at 620 nm wavelength. The concentration of reducing sugar was calculated from graph plotted using glucose as a standard.

2.5.4. Catalase activity (CAT) assay

Activities of catalase enzyme were measured as described by Chance and Maehly (1955). Fresh leaf material (1 g) was crushed in 5 ml of ice-cold 50 mM potassium phosphate buffer (pH 7) and 1% (w/v) polyvinylpyrrolidone (PVP). The homogenates were centrifuged at 10,000 g (4°C) for 10 min. The leaf extracts were used for the quantification of soluble protein content using Bradford method and analysis of catalase activity. Catalase activity was measured in a reaction mixture (3 ml) containing 100 mM Na_2HPO_4 buffer pH 6.8 (2 ml), 30 mM H_2O_2 (0.5 ml) and 0.5 ml enzyme. For assaying CAT activity, the decomposition of H_2O_2 was followed by decline in the absorbance at 240 nm as catalase enzyme catalyzes the reaction (3):

$$2H_2O_2 \rightarrow 2H_2O + O_2 \quad (3)$$

CAT activity was determined by following the consumption of H_2O_2 (extinction coefficient, 39.4 M^{-1} cm^{-1}) at 240 nm over a 3 min interval.

2.5.5. Peroxidase activity

Peroxidase activity was estimated following the method of Hammerschmidt et al. (1982). One gram of leaf was homogenized in ice cold 0.1 M phosphate buffer (5.0 ml; pH 6.0) at 4 °C. It was centrifuged at 16000 rpm at 4°C for 15 min. The supernatant was used as enzyme source. The reaction mixture contained 0.05 M pyrogallol (3.0 ml), enzyme extract (100 μl) and 1% H_2O_2 (0.5 ml). The absorbance was recorded at every 30s interval for 3 min at 420 nm. The enzymatic activity was expressed as Units/ml enzyme.

2.6. Statistical analysis

The data of the antagonistic potential of endophytes was analyzed and transformed (arc sine) using MS-Excel©. Multiple comparisons were

performed for analyzing the plant growth promotion and antioxidative enzyme data, wherein Duncan Multiple Range test (DMRT) was applied for endophytes as treatments using web resource Web Agri Stat Package (WASP v2.0). Molecular analysis of ITS sequences was done using web resource NCBI-BLASTN (https://blast.ncbi.nlm.nih.gov/) to identify the fungal endophytes.

3. Results

3.1. Identification of endophytic fungi and their antagonistic potential

Fungal endophytes were identified morphologically through their spore characters and also through universal ITS 1 and ITS 4 markers. The ITS sequences of the endophytes were submitted to NCBI *GenBank* database. In total, eight endophytes were tested for antagonisity against two pathogens *viz.*, *C. cassiicola* and *F. solani*. Among them, four belonged to *Diaporthe* genus (CFS-5, CFL-34, CEL-41 and CEL-48) and two were *Macrophomina phaseolina* (CFR-1 and CFL-27). One isolate each of *Fusarium solani* (CEP-20) and *Daldinia eschscholtzii* (M_1-4) were also identified.

Under dual culture technique, fungal endophytes showed antagonism to both the pathogens tested with colony inhibition percentage (Table 1) ranging from 52-66% against *C. cassiicola* and 45–50% against *F. solani*, except CFL-27 and CEP-20, wherein inhibition percentage was 20.82% and 36.14% respectively against the former. While, maximum inhibition was shown by CFR-1 (66.16%) followed by CEL-48 (64.24%) against *C. cassicola*. Further, remaining three isolates of *Diaporthe* were also efficient in inhibiting the pathogen (63.6% for CFL-34). Likewise, the antagonisity of endophytes against pathogenic *F. solani* was also encouraging, with inhibition as high as 52.8% in CFL-34 followed by CFR-1 (50.31%). *Diaporthe longicolla* (CEL-48) too was effective against *F. solani* as in case of *C. cassiicola*.

3.2. Effect of endophyte treatment on germination percentage and seed vigor index in cotton

Two cotton cultivars *viz.*, Suraj and Phule Dhanwantary were treated with spore suspension of eight different fungal endophytes and it was evident from the germination paper study that, biopriming of seeds with endophytes had positive effect on seed germination. Germination count for each endophyte and cultivar was recorded after 10 days and germination percentage was calculated (Figure 2). As compared to control, seed germination was high in all the endophyte treatments in both the cultivars. Seed germination was highest for CFL-34 and CFR-1 (93.33%) in Suraj followed by CEP-20 (90.0%). Whereas, CEP-20 and CFL-27 resulted in high seed germination (90.0% and 83.33% respectively) as compared to control (which was only 46.67%) in Phule Dhanwantary.

Table 1. Antagonistic potential of fungal endophytes against *Corynespora cassiicola* and *Fusarium solani*.

SN	Fungal endophyte isolate	Colony inhibition (%)	
		Corynespora cassiicola	*Fusarium solani*
1	*Macrophomina phaseolina* (CFL-27)	20.82 (26.44)	15.53 (23.15)
2	*Diaporthe* sp. (CFL-34)	63.60 (52.93)	52.80 (46.63)
3	*Diaporthe longicolla* (CEL-41)	52.11 (46.24)	32.30 (34.65)
4	*Diaporthe longicolla* (CEL-48)	64.24 (53.30)	49.69 (44.84)
5	*Fusarium solani* (CEP-20)	36.14 (36.96)	27.95 (31.79)
6	*Diaporthe melonis* (CFS-5)	54.66 (47.70)	28.57 (32.33)
7	*Macrophomina phaseolina* (CFR-1)	66.16 (54.48)	50.31 (45.20)
8	*Daldinia eschscholtzii* (M_1-4)	53.38 (46.97)	45.34 (42.35)
	CD (0.01)	8.79	5.34
	SEm±	1.01	0.61

Figures in parentheses are Arc sign transformed values.

Among others, CFS-5 and CEL-41 were also responsible for higher germination in Phule Dhanwantary.

Seed vigor is another important quality parameter which determines the performance. Seed vigor index was determined by considering germination percentage and seedling length (Online resource 2). It was observed that, seed vigor index was high in endophyte bio-primed cotton plants as compared to control plants (Figure 3). It was highest in CFR-1 (14646) followed by CFS-5 (13868) in case of Suraj; while it was highest in CEP-20 followed by M_1-4 in Phule Dhanwantary (10770 and 9619 respectively). Simultaneously, endophytes CFL-34 and M_1-4 too had high vigor index in Suraj, but CFL-27 and CEL-41 were found prominent in Phule Dhanwantary. Overall, the results suggested that biopriming with endophytes has certainly improved the seed vigor index in comparison with the control.

3.3. Effect of seed biopriming on germination of non-host crops

Seed germination was enhanced in all the endophyte bioprimed non-host crops as compared to control (Figure 4). Among all the endophytes, *Diaporthe longicolla* (CEL-48) showed the highest germination percentage in all the crops (93.33%) followed by CEL-41 and M_1-4. Significant increase in germination percentage over control was visible in bioprimed wheat and chickpea crops, suggesting the role of endophytes.

3.4. Effect of biopriming on plant growth promotion

3.4.1. Total soluble protein

Total soluble protein was examined to study the effect of endophyte priming on plant growth in different crop plants. A visible increase was evident in the primed samples of all the crops as compared to their control samples. The overall protein content was more in cowpea and redgram as against cotton, wheat and chickpea (Figure 5). The percent increase under different treatments over control ranged from 10.6 to 30.8 % in cotton, 10.1–32.8 % in chickpea, 23.2–69.1 % in wheat, and 28.4–58.3 % in cowpea (Table 2).

3.4.2. Total sugar and reducing sugar

Total soluble sugar and reducing sugar are the key parameters to assess the plant growth promotion. Total sugar content ranged from 5.46 to 7.54 mg/g F.W in cotton, 2.84–6.55 mg/g F.W in chick pea, 4.63–8.64 mg/g F.W in wheat, 1.52–5.06 mg/g F.W in cowpea and 4.73–7.67 mg/g F.W in Redgram (Table 3). Compared to their respective controls, all the crops under investigation had higher sugar content in endophyte primed treatments except cowpea treated with CEL-41. We could observe the highest sugar content in CFL-34 treated wheat samples. No treatment in common was found to relate the highest sugar content with specific endophyte priming for all the crops.

Reducing sugar content followed the same trend as of total soluble sugar. Though, the variation among treatments was not much, endophyte primed samples of all the crops including cotton were having more reducing sugar than their respective controls. The content was in the range of 0.88 mg/g F.W (cowpea control) to 2.49 mg/g F.W (CEL-41 treated cotton).

3.5. Biopriming improves the antioxidant system in host and non-host crops

3.5.1. Catalase activity

Host as well as non-host crops demonstrated an increase in catalase activity in bioprimed leaf samples, when compared to control. Though, this increase was common to all treatment in all the crops, CFS-5 resulted in decline of catalase activity except in chickpea. Specific activity of catalase ranged from 0.42 to 1.90 μmol/min/mg protein in cotton, 0.96–3.96 μmol/min/mg protein in chickpea, 1.99–4.32 μmol/min/mg protein in wheat, 0.44–2.82 μmol/min/mg protein in cowpea and 0.19–3.41 μmol/min/mg protein in redgram (Figure 6). Highest activity

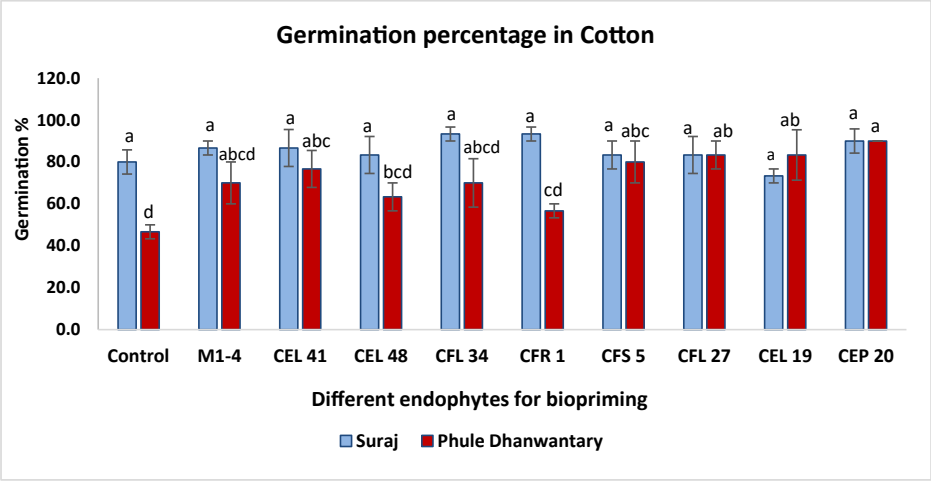

Figure 2. Germination percentage in different endophyte bioprimed cotton cultivars Suraj and Phule Dhanwantary. Error bars are SE of the mean (n = 3). Different letters above the error bars indicate statistically significant difference ($P \leq 0.05$).

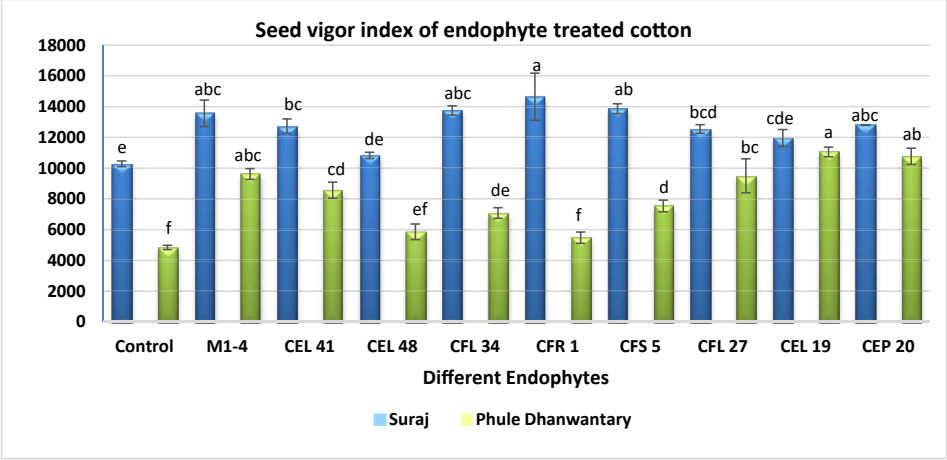

Figure 3. Seed vigor index in cotton cultivars Suraj and Phule Dhanwantary, bioprimed with eight different fungal endophytes. Different letters above the error bars indicate statistically significant difference ($P \leq 0.05$).

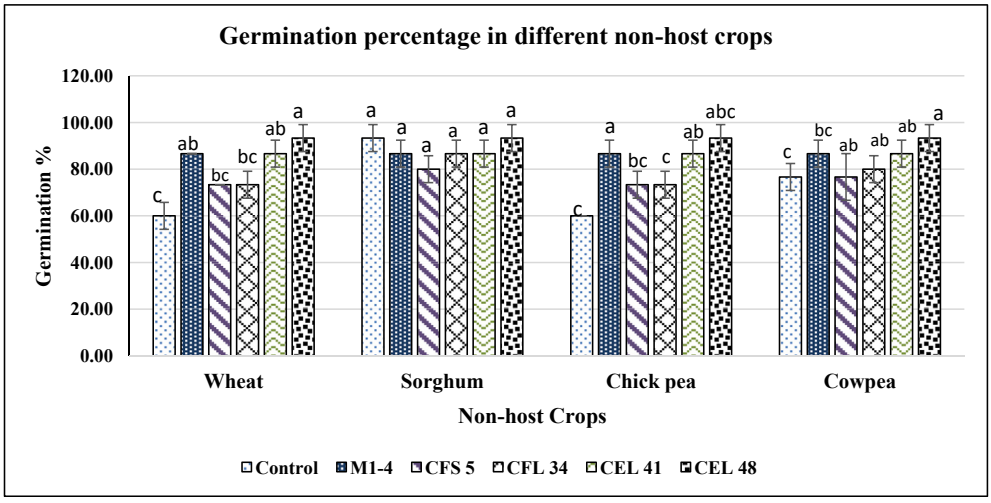

Figure 4. Germination percentage in different non-host crops bioprimed with cotton fungal endophytes. Error bars are SE of the mean (n = 3). Different letters above the error bars indicate statistically significant difference ($P \leq 0.05$).

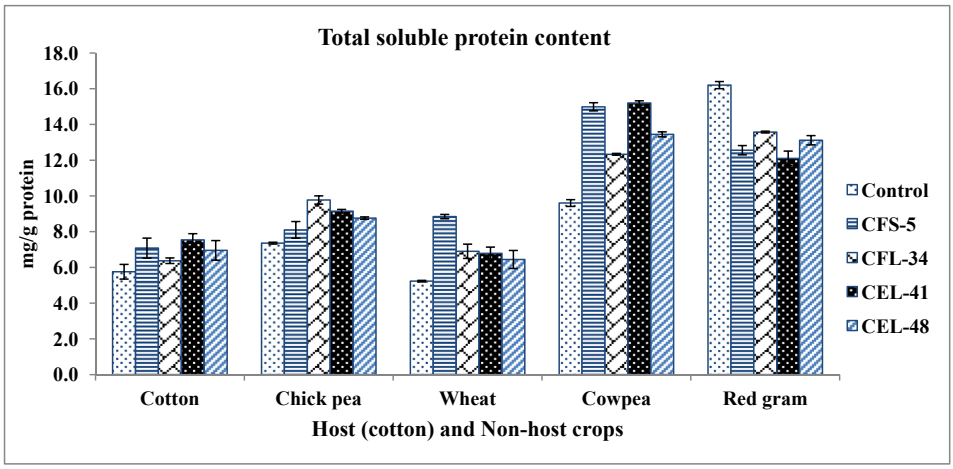

Figure 5. Total soluble protein content in leaves of host and non-host crops under different endophyte treatments. Error bars are SE of the mean (n = 3). There was no significant difference among the treatments.

Table 2. Per cent increase in total soluble protein of endophyte bio-primed host and non-host crops.

	Cotton (mg/g)	% Increase	Chick pea (mg/g)	% Increase	Wheat (mg/g)	% Increase	Cowpea (mg/g)	% Increase	Red gram (mg/g)	% Increase
Control	5.76	-	7.36	-	5.23	-	9.60	-	16.20	-
CFS-5	7.08	**22.95**	8.10	**10.10**	8.84	**69.13**	14.99	**56.10**	12.56	**-22.49**
CFL-34	6.37	**10.67**	9.77	**32.82**	6.90	**31.97**	12.33	**28.42**	13.57	**8.08**
CEL-41	7.54	**30.89**	9.14	**24.27**	6.79	**29.78**	15.20	**58.33**	12.10	**-10.84**
CEL-48	6.95	**20.72**	8.76	**19.03**	6.44	**23.22**	13.44	**40.03**	13.11	**8.38**

Table 3. Total soluble sugar and reducing sugar (±SE) in leaves of host and non-host crops under different endophyte treatments.

Treatments	Cotton		Chick pea		Wheat		Cowpea		Red gram	
	Total Sugar (mg/g FW)	Reducing Sugar (mg/g FW)	Total Sugar (mg/g FW)	Reducing Sugar (mg/g FW)	Total Sugar (mg/g W)	Reducing Sugar (mg/g W)	Total Sugar (mg/g W)	Reducing Sugar (mg/g FW)	Total Sugar (mg/g W)	Reducing Sugar (mg/g W)
Control	5.46 ± 0.23	1.736 ± 0.25	2.84 ± 0.40	1.57 ± 0.35	4.63 ± 0.32	1.55 ± 0.17	3.20 ± 0.26	0.880 ± 0.39	4.73 ± 0.27	1.691 ± 0.18
CFS-5	6.71 ± 0.29	2.101 ± 0.38	4.91 ± 0.27	1.64 ± 0.15	6.6 7 ± 0.52	1.65 ± 0.27	2.19 ± 0.32	1.285 ± 0.19	7.00 ± 0.45	1.701 ± 0.23
CFL-34	5.92 ± 0.89	2.411 ± 0.42	6.55 ± 0.17	1.680 ± 0.14	8.64 ± 0.25	1.75 ± 0.38	3.26 ± 0.21	1.547 ± 0.82	7.67 ± 0.36	1.767 ± 0.15
CEL-41	7.54 ± 0.18	2.488 ± 0.31	6.53 ± 0.21	1.633 ± 0.23	7.50 ± 0.26	1.64 ± 0.25	1.52 ± 0.35	1.385 ± 0.71	5.94 ± 0.24	1.712 ± 0.27
CEL-48	5.58 ± 0.11	1.930 ± 0.21	4.53 ± 0.16	1.791 ± 0.12	5.83 ± 0.63	1.63 ± 0.19	5.06 ± 0.17	1.557 ± 0.26	7.31 ± 0.55	1.716 ± 0.62

was reported in CEL-48 primed wheat, whereas lowest catalase activity was observed in CFS-5 treated redgram.

3.5.2. Peroxidase activity

Peroxidase activity was found to be comparable in host as well as non-host crops. Compared to their respective controls, bioprimed samples of all the crops had higher peroxidase activity, except CFL-34 treated wheat and redgram (Figure 7). CEL-48 treated redgram showed highest peroxidase activity (2.63 Units/ml enzyme), whereas chickpea control had lowest peroxidase activity (0.88 Units/ml enzyme).

4. Discussion

Endophytes are key components of a plant's microbiome that play a crucial role in plant-pathogen, plant-herbivore and plant-nematode interactions through various mechanisms. Studies as far back as 1920s have recorded the identification of several fungal endophytes from different cotton tissues and those have been reported to be endophytes across many plant species (Crawford 1923; Palmateer et al., 2004; Ek-Ramos et al., 2013). Previous work on endophytes in cotton is mostly limited to entomopathogenic fungi such as *Beauveria bassiana, Purpureocillium lilacinum, Metarrhizium anisopliae* etc. Some of the reports indicate that cotton endophytes have been utilized to manage the soil borne pathogens, Verticillium wilt in particular (Yuan et al., 2017) and recently we have also reported the antagonism of fungal endophytes isolated from *Gossypium arboreum* against *C. cassiicola* and *F. solani* (Hiremani et al., 2020).

In this study, eight fungal endophytes identified based on their spore characters and ITS sequence analysis were found promising through their antagonistic activity against the test pathogens (Table 1). The mechanism of antagonism was mostly through competition for space and nutrients in the confrontation assay. Two endophytes, *Macrophomina phaseolina* (CFR-1) and *Diaporthe longicolla* (CEL-48), were highly efficient against both the pathogens *in vitro* and inhibited their colony growth (Table 1). Though all the four isolates of *Diaporthe* were efficient and inhibited more than 52% of growth in *C. cassiicola*, it was not the same against *F. solani*. Because, the percent colony inhibition in *F. solani* by these four isolates was variable from 28-52%. This may be due to the fact that *F. solani* is fast growing as compared to *Diaporthe*. Surprisingly, another isolate of *M. phaseolina* (CFL-27) was not so effective in inhibiting the colony growth of both the pathogens unlike CFR-1. Our findings are in concurrence with the previous reports where endophytes have been utilized for biological control of plant pathogens. For instance, four endophytic fungi *viz.*, *Penicillium simplicissimum* (CEF-818), *Leptosphaeria*

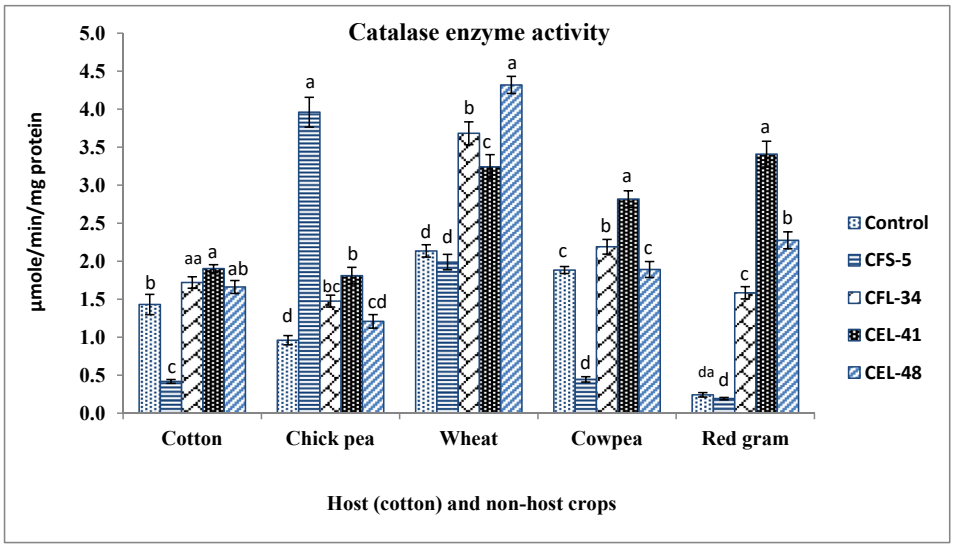

Figure 6. Catalase activity in leaves of host and non-host crops under different endophyte treatments. Error bars are SE of the mean (n = 3). Different letters above the error bars indicate statistically significant difference ($P \leq 0.05$).

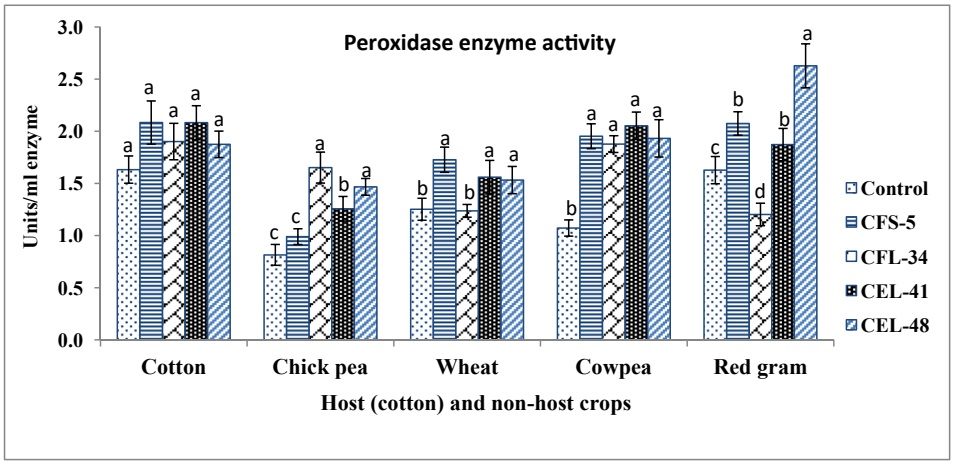

Figure 7. Peroxidase activity in leaves of host and non-host crops under different endophyte treatments. Error bars are SE of the mean (n = 3). Different letters above the error bars indicate statistically significant difference ($P \leq 0.05$).

sp (CEF-714), *Talaromyces flavus* (CEF-642) and *Acremonium* sp (CEF-193), isolated from cotton roots were assessed against wilt causing *Verticllium dahliae* strain Vd080 and it was found that the control efficacy of these endophytes ranged from 26-67% at 25 dpi (Yuan et al., 2017). In another study, endophytic *Fusarium solani* CEF559 was also found effective *in vitro* by inhibiting colony growth and sporulation (by 75% and 80% respectively) of wilt inciting pathogen *V. dahliae* (Wei et al., 2019). However, the endophytic *F. solani* (CEP-20) identified in our study (Table 1) was not as effective against the test pathogens. Moreover, in our previous study, we have screened 23 fungal endophytes from *desi* cotton (*G. arboreum* L.) and found that endophytes were efficient in inhibiting up to 49% of the colony growth in *C. cassiicola* under *in vitro* conditions (Hiremani et al., 2020).

Utilization of endophytes for seed biopriming has scores of benefits in the field of agriculture and seed technology in particular. Available evidence has shown the positive influence of biopriming on seed quality parameters and plant growth promotion. But, studies on seed priming with endophytes or the effect of biopriming on antagonisity, seed germination and other growth parameters in cotton are lacking and/or are very few in number. On the contrary, seed biopriming studies are mostly limited to popular biocontrol agents such as *Trichoderma viride*, *T. harzianum* and *Pseudomonas fluorescens* being used for biopriming in many of the crops (Aamir et al., 2019; Ferrigo et al., 2019; Singh et al., 2020a; Swain et al., 2021).

The findings of the seed germination study in endophyte bio-primed cotton cultivars revealed that seed germination was enhanced upon priming in both the cultivars Suraj and Phule Dhanwantary (Figure 2). Germination percentage of cotton seeds were significantly different for all the treatments over control. Two endophytes, CFL-34 and CFR-1 had highest seed germination in Suraj whereas, endophytic *F. solani* isolate CEP-20 (90%) had shown highest germination as compared to others in Phule Dhanwantary, thus suggesting it is not a latent pathogen but a beneficial endophyte. Earlier reports corroborate the findings of our study, e.g. Lin et al. (2007) observed that the germination of rice seeds infected with endophytic *Phomopsis* sp. was significantly higher as against endophyte free plants. In another study, fourteen endophytes were tested for their efficacy on seed germination in rice and it was found that, *Penicillium citrinum* showed maximum seed germination, whereas seeds treated with *Cladosporium cladosporioides* exhibited highest shoot length (Lalngaihawmi et al., 2018). In a recent report, biopriming with *Trichoderma* strains reduced the mean germination time, increased the seedling vigor and chlorophyll content in two rice varieties (Swain et al.,

2021). Therefore, biopriming with endophytes, not only antagonists like *Trichoderma*, has proven to be a better tool in enhancing the germination in many crops like rice, maize and now in cotton through the findings of this experiment (Figure 2).

Endophytes are ubiquitous but their specificity to hosts is debatable, *e.g.* Clavicipitaceous endophytes are distributed in grasses and sedges (Leuchtmann 1992), whereas *Piriformospora indica* and many other dark septate endophytes (DSEs) have been popularly known to colonize and promote the growth of many non-host plants. The role of DSE in enhancing the stress tolerance of plants is evident in several studies. According to a recent study, nine DSEs isolated from a super-xerophytic shrub, *Gymnocarpos przewalskii*, were found effective against *Ammopiptanthus mongolicus* under drought condition, when applied as non-host DSE, wherein they were found to have significantly positive effects on plant branch number, potassium and calcium content and overall plant biomass (Li et al., 2018). Similarly, another study on DSE revealed that inoculation by non-host DSE strains (*Phialophora* sp., *Knufia* sp., *Leptosphaeria* sp. and *Embellisia chlamydospora*) isolated from other desert plants benefited *Hedysarum scoparium* by improving the root biomass, total biomass, nutrients concentration, and antioxidant enzyme activities of host plants under drought conditions (Li et al., 2019). This study too found that, biopriming with cotton endophytes in host as well as non-host crops showed remarkable growth promotion characteristics (Figure 5). Our results indicated increase in total soluble protein ranging from 10.1 up to 69.1% in bioprimed sample in different non-host crops (Table 2). These are comparable to the study where, *P. aeruginosa* MF-30 primed seeds of maize significantly improved the total soluble protein content in the maize plants (Singh et al., 2020a). When mixture of strains (*Pseudomonas fluorescens* + *Rhizobium phaseoli*)) were used to bioprime the mung bean seeds, total soluble protein as well as total grain yield, even under drought stress, was found to be enhanced (Nawaz et al., 2021). Quantitative estimation of biomolecules (protein, sugar, lipids *etc*.) help in assessing the real impact of bio-agents/biopriming treatments in plants. Almost all the bio-primed host and non-host plants observed higher amount of total soluble sugar content. Similar trend was seen in case of reducing sugar which was higher in the bio-primed leaves of host and non-host plants. Plants bio-primed with *Pseudomonas geniculate* were able to overproduce the sugar as compared to unprimed samples in maize (Singh et al., 2020b). Our findings were also in agreement with the previous studies where better carbohydrate production and accumulation in PGR-bioprimed samples have been documented (Podile and Kishore 2006; Hayat et al., 2012).

In order to determine the potential of biopriming with cotton fungal endophytes in eliciting antioxidant response, activity of antioxidant enzymes; catalase and peroxidase, was evaluated for host and non-host crops. In the present study, seed biopriming with different endophytes enhanced the catalase activity by almost two to four times compared to control (Figure 6). Similar trend has been reported in earlier studies where seed priming with either endophytic bacteria, *Trichoderma* or some *Rhizobacteria* resulted in increased catalase activity (Chakraborty et al., 2011; Swain et al., 2021). Peroxidase activity was also higher in all the bioprimed samples across the crops (Figure 7) but the variations among the treatments were not as much as of catalase enzyme (Rajput et al., 2019; Swain et al., 2021).

We could find one or two treatments which were not at par even with control while assessing growth promotion parameters and antioxidant potential. Cowpea treated with CEL-41 reduced the total sugar content, whereas CFS-5 primed chickpea and CFL-34 primed wheat and red gram reported lower catalase and peroxidase activity than control. Since, we evaluated all the crops at 60 days after sowing (DAS), it is possible that priming with these endophytes elicits the response only at early stage of the crops. Further, no treatment or fungal endophyte in common was found best for all the crops. It suggests that the compatibility of each endophyte may differ with change of crop plant.

5. Conclusion

This study was unique by way of biopriming the seeds with fungal endophytes and to investigate the effect of biopriming on seed germination, growth promotion parameters like total soluble protein, total sugar and also antioxidative system in cotton. Additionally, the endophytes proved their potential in biocontrol efficacy (up to 66%) against two cotton pathogens. Most importantly, the effect of cotton endophytes on other non-host crops like wheat, cowpea, red gram and chick pea was also found to benefit them for germination, growth promotion and antioxidative system. An increase in total soluble protein content as high as 69% was visible in wheat bioprimed with CFS-5 and 58% in cowpea bioprimed with CEL-48. Therefore, endophyte biopriming plays an important role in healthy plant growth and suppressing the pathogen growth; thus, may be utilized as potential technology in advancing a dynamic and sustainable agriculture.

Declarations

Author contribution statement

Pooja Verma: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Neelakanth S. Hiremani:: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Shailesh P. Gawande: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Satish K. Sain: Analyzed and interpreted the data; Wrote the paper.

Dipak T. Nagrale: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Nandini G. Narkhedkar; Y. G. Prasad: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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References

Aamir, M., Kashyap, S.K., Zehra, A., Dubey, M.K., Singh, V.K., Ansari, W.A., Upadhyay, R.S., Singh, S., 2019. *Trichoderma erinaceum* biopriming modulates the WRKYs defense programming in tomato against the *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) challenged condition. Front. Plant Sci. 10, 911.

Abdul-Baki, A., Anderson, J.D., 1973. Vigor determination in Soybean seed by multiple criteria. Crop Sci. 13, 630–633.
Bell, D.K., Wella, H.D., Markham, C.R., 1982. *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology 72, 379–382.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Chakraborty, U., Roy, S., Chakraborty, A.P., Dey, P., Chakraborty, B., 2011. Plant growth promotion and amelioration of salinity stress in crop plants by a salt-tolerant bacterium. Recent Res. Sci. Technol. 3, 61–70.
Chance, B., Maehly, A.C., 1955. Assay of catalases and peroxidases. Meth. Enzymol. 2, 764–775.
Clay, K., 1987. Effects of fungal endophytes on the seed and seedling biology of *Lolium perenne* and *Festuca arundinacea*. Oecologia 73, 358–362.
Crawford, R.F., 1923. Fungi isolated from the interior of cotton seed. Phytopathology 13, 501–503.
Ek-Ramos, M.J., Zhou, W., Valencia, C.U., Antwi, J.B., Kalns, L.L., Morgan, G.D., Kerns, D.L., Sword, G.A., 2013. Spatial and temporal variation in fungal endophyte communities isolated from cultivated cotton (*Gossypium hirsutum*). PLoS One 8, 1–13.
Entesari, M., Sharifzadeh, F., Ahmadzadeh, M., Farhangfar, M., 2013. Seed biopriming with *Trichoderma* species and *Pseudomonas fluorescense* on growth parameters, enzymes activity and nutritional status of soybean. Int. J. Agron. Plant Prod. 4, 610–619.
Ferrigo, D., Mondina, M., Ladurnerb, E., Fiorentinib, F., Causina, R., Raiolaa, A., 2019. Effect of seed biopriming with *Trichoderma harzianum* strain INAT11 on *Fusarium* ear rot and *Gibberella* ear rot diseases. Biol. Control 147, 104286.
Hammerschmidt, R., Nuckles, E.M., Kuć, J., 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol. Plant Pathol. 20, 73–82.
Hayat, R., Ahmed, I., Sheirdil, R.A., 2012. An overview of plant growth promoting rhizobacteria (PGPR) for sustainable agriculture. In: Ashraf, M., Öztürk, M., Ahmad, M., Aksoy, A. (Eds.), Crop Production for Agricultural Improvement. Springer, Dordrecht.
Hiremani, N.S., Verma, P., Gawande, S.P., et al., 2020. Antagonistic potential and phylogeny of culturable endophytic fungi isolated from *desi* cotton (*Gossypium arboreum* L.). South Afr. J. Bot. 134, 329–335.
Jaber, L.R., 2018. Seed inoculation with endophytic fungal entomopathogens promotes plant growth and reduces crown and root rot (CRR) caused by *Fusarium culmorum* in wheat. Planta 248, 1525–1535.
Jaber, L.R., Enkerli, J., 2016. Effect of seed treatment duration on growth and colonization of *Vicia faba* by endophytic *Beauveria bassiana* and *Metarhizium brunneum*. Biol. Control 103, 187–195.
Kumar, M., Pant, B., Mondal, S., Bose, B., 2016. Hydro and halopriming: influenced germination responses in wheat var HUW-468 under heavy metal stress. Acta Physiol. Plant. 38, 217.
Kumar, A., Droby, S., White, J.F., Singh, V.K., Singh, S.K., Zhimo, V.Y., Biasi, A., 2020. Endophytes and seed priming: agricultural applications and future prospects. In: Kumar, A., Radhakrishnan, E.K. (Eds.), Microbial Endophytes. Woodhead Publishing, pp. 107–124.
Lalngaihawmi, Banik, S., Chakruno, P., Khatemenla, 2018. Effect of rice fungal endophytes on seed germination and seedling growth of rice. Int. J. Curr. Microbiol. App. Sci. 7, 3653–3663.
Leuchtmann, A., 1992. Systematics, distribution, and host specificity of grass endophytes. Nat. Toxins 1 (3), 150–162.
Li, Z.F., Wang, L.F., Feng, Z.L., Zhao, L.H., Shi, Y.Q., Zhu, H.Q., 2014. Diversity of endophytic fungi from different Verticillium-wilt resistant *Gossypium hirsutum* and evaluation of antifungal activity against *Verticillium dahliae in vitro*. J. Microbiol. Biotechnol. 24, 1149–1161.
Li, X., He, X., Hou, L., et al., 2018. Dark septate endophytes isolated from a xerophyte plant promote the growth of *Ammopiptanthus mongolicus* under drought condition. Sci. Rep. 8, 7896.
Li, X., He, X., Zhou, Y., Hou, Y., Zuo, Y., 2019. Effects of dark septate endophytes on the performance of *Hedysarum scoparium* under water deficit stress. Front. Plant Sci. 10, 903.
Lin, Y.Z., Chao, D.C., Xia, L., Shuang, T.L., Xiang, W.X., 2007. Extensive host range of an endophytic fungus affects the growth and physiological functions in rice (*Oryza sativa* L.). Symbiosis 43, 21–28.
Mahmood, A., Kataoka, R., 2019. Application of endophytes through seed priming. In: Hasanuzzaman, M., Fotopoulos, V. (Eds.), Priming and Pretreatment of Seeds and Seedlings. Springer, Singapore.
McDonald, M.B., 2000. Seed priming. In: Black, M., Bewley, J.D. (Eds.), Seed Technology and its Biological Basis. Sheffield Academic Press, Sheffield, pp. 287–325.
Nawaz, H., Hussain, N., Ahmed, N., Javaiz, A.L., 2021. Efficiency of seed biopriming technique for healthy mungbean productivity under terminal drought stress. J. Integr. Agric. 20, 87–99.
Nelson, N., 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153, 375–380.
Palmateer, A.J., McLean, K.S., Morgan-Jones, G., van Santen, E., 2004. Frequency and diversity of fungi colonizing tissues of upland cotton. Mycopathologia 157, 303–316.
Podile, A.R., Kishore, G.K., 2006. Plant growth promoting rhizobacteria. In: Gnanamanickam, S.S. (Ed.), Plant Associated Bacteria. Springer, Amsterdam, pp. 195–230.
Rajput, R.S., Singh, P., Singh, J., Vaishnav, A., Ray, S., Singh, H.B., 2019. *Trichoderma* mediated seed biopriming augments antioxidant and phenylpropanoid activities in tomato plant against *Sclerotium rolfsii*. J. Pharmacogn. Phytochem. 8, 2641–2647.
Singh, S., Singh, U.B., Malviya, D., Paul, S., Sahu, P.K., Trivedi, M., Paul, D., Saxena, A.K., 2020a. Seed biopriming with microbial inoculant triggers local and systemic defense responses against *Rhizoctonia solani* causing banded leaf and sheath blight in maize (*Zea mays* L.). Int. J. Environ. Res. Publ. Health 17, 1396.
Singh, S., Singh, U.B., Trivedi, M., Sahu, P.K., Paul, S., Paul, D., Saxena, A.K., 2020b. Seed biopriming with salt-tolerant endophytic *Pseudomonas geniculata*-modulated biochemical responses provide ecological fitness in maize (*Zea mays* L.) grown in saline sodic soil. Int. Env. Res. Pub. Health 17, 253.
Swain, H., Adak, T., Mukherjee, A.K., Sarangi, S., Samal, P., Khandual, A., Jena, R., Bhattacharyya, P., Naik, S.K., Mehetre, S.T., Baite, M.S., Kumar, M.S., Zaidi, N.W., 2021. Seed biopriming with *Trichoderma* strains isolated from tree bark improves plant growth, antioxidative defense system in rice and enhance straw degradation capacity. Front. Microbiol. 12, 633881.
Thimmaiah, S.R., 2012. Standard Methods of Biochemical Analysis. Kalyani Publishers, New Delhi, India.
Van Hulten, M., Pelser, M., Van Loon, L.C., Pieterse, C.M., Ton, J., 2006. Costs and benefits of priming for defense in *Arabidopsis*. Proc. Nat. Acad. Sci. 3, 5602–5607.
Vinale, F., Sivasithambaram, K., Ghisalberti, L.E., Marra, R., Woo, S.L., Lortio, M., 2008. *Trichoderma*-plant pathogen interactions. Soil Biol. Biochem. 40, 1–10.
Waller, F., Achatz, B., Baltruschat, H., et al., 2005. The endophytic fungus *Piriformospora indica* reprograms barley to salt stress tolerance, disease resistance, and higher yield. Proc. Natl. Acad. Sci. U.S.A. 102, 13386–13391.
Wei, F., Zhang, Y., Shi, Y., Feng, H., Zhao, L., Feng, Z., Zhu, H., 2019. Evaluation of the biocontrol potential of endophytic fungus *Fusarium solani* CEF559 against *Verticillium dahliae* in cotton plant. BioMed Res. Int. 3187943.
White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. (Eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, New York, pp. 315–322.
Wicklow, D.T., Roth, S., Deyrup, S.T., Gloer, J.B., 2005. A protective endophyte of maize: *Acremonium zeae* antibiotics inhibitory to *Aspergillus flavus* and *Fusarium verticilloides*. Mycol. Res. 109, 610–618.
Yuan, Y., Feng, H., Wang, L., Li, Z., Shi, Y., Zhao, L., et al., 2017. Potential of endophytic fungi isolated from cotton roots for biological control against Verticillium wilt disease. PLoS One 12, e0170557.