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Research Article

Seed priming with endophytes on physiological, biochemical and antioxidant activity of hybrid maize (*Zea mays* l.) COH (M) 8 seeds

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Abstract
Endophytes are important microorganisms that enhance the plant's stability through a symbiotic relationship, without any harmful effects and symptoms in the host plant. To study the effect of endophytes on overall performance of COH(M)8 hybrid maize seeds, the present study was conducted with different endophytic seed priming for 12 hrs duration with *Beauveria bassiana* @ 5% (T_2), *Metarhizium anisopliae* @ 5% (T_3) *and Bacillus subtilis* @ 8% (T_4) along with hydro priming (T_1) and untreated control (T_0). The seed priming treatments with all the above three endophytes enhanced the seed quality parameters, among which *M. anisopliae* @ 5% (T_3) registered maximum increase of germination (4.34%), shoot length (20.73%), root length (15.04%), dry matter production (15.22%) and vigour index (22.68%) over control. Similarly, the seeds primed with *M. anisopliae* @ 5% (T_3) recorded the highest value of dehydrogenase activity (0.441 OD value), α- amylase activity (2.06 mg maltose min^{-1}) and antioxidant activity *viz.,* catalase (1.55 μmol H_2O_2 min^{-1} g^{-1} protein) and peroxidase (0.87 U mg^{-1}protein min^{-1}). Results of this study revealed that the endophytes can enhance overall the seed quality in maize.

Keywords: Endophytes, Maize, Seed priming, Seed germination

INTRODUCTION

Endophytes are endosymbionts that are integral components of a plant micro-ecosystem, which is associated with the plant throughout its life cycle without inducing any disease. They perform symbiotic functions, including production of secondary metabolites or synthesis of signalling molecules that function as both internal and external stimuli during mutualistic interactions (Tidke *et al.,* 2018). It produces biologically active metabolites like plant growth promotors, antioxidants, antimicrobial activity, insecticides and antibiotics (Strobel, 2018; Khalil *et al.,* 2021). These endophytes, which include fungi, bacteria, actinomycetes, protozoa or algae have vital role in improving the fitness of plants. Symbiotic endophytes are present in all plant parts, such as leaves, stems, branches, roots, root hairs, twigs, flowers and seeds (White *et al*., 2015; Gond *et al*., 2015).

Fungal endophytes are generally known as fungi that colonize the internal tissues of plants for their part of or all of their lifecycle (Wilson, 1995). Fungal endophytes may exhibit systemic colonization (Gurulingappa *et al*., 2010) and be confined in plant parts (Yan *et al*., 2015). It performs various roles such as symbiotic and ecological functions (Rodriguez *et al*., 2009) that benefit plants, including resistance against both biotic and abiotic stresses. Among the fungal endophytes, *B. bassiana* has been found naturally as an endophyte in several plant species and can also be artificially introduced into different crops viz., maize (Gayathri *et al*., 2020), cotton (Amutha, 2021) and tomato (Deb and Dutta, 2021). Among the fungal endophytes, *Metarhizium* play an important role in plant growth. More recently, enormous studies have reported the presence of diverse species of *Metarhizium*, such as *M. anisopliae* and *M. robertsii,* as endophytes in different plant species (Greenfield *et al*., 2016).

Endophytic bacteria are commonly called plant growth promoting rhizobacteria (PGPR). These are a subset of rhizobacteria that can colonize their plant hosts (Reinhold-Hurek and Hurek, 1998). In PGPR, it exhibits either/or both direct and indirect ways to promote plant growth and increase plant stress tolerance. Among the bacterial endophytes, the *Bacillus* genus is the most promising bacteria. Several species of this genus have been classified as plant growth promoters and biological control agents, among which *B. subtilis* stands out (Khan *et al*., 2018).

The significance of sustainable agricultural production is hidden in the use of quality seeds. Seed is the most crucial and vital input for enhancing yield and productivity. One of the most important aspects of quality seeds is the production of pest and disease-free seeds which will enhance the vigour, viability and optimum field stand. Seed enhancement techniques were used to improve germination and seedling growth. Seed priming is one of the important quality enhancement techniques. During seed priming, the amount of water absorption is controlled to prevent radicle emergence but only allow necessary metabolic activities for seed germination (Heydecker *et al.,* 1973; Paparella *et al.,* 2015). It enhances the uniformity, establishment and seedling vigour of various crops. During seed priming with endophytes, it enters into the seeds and adapt to the existing conditions. It is capable of fixing nitrogen, solubilizing phosphorus, enhancing phosphorus uptake and production of siderophores and plant hormones such as auxin, abscisic acid, ethylene, GA_3 and IAA, which are important for plant growth and development (Xu *et al*., 2014). Hence, this study was conducted to evaluate the effect of endophytes on seed germination, seedling growth and biochemical and antioxidant activity of maize under laboratory conditions.

MATERIALS AND METHODS

Collection of experiment materials

The freshly harvested and genetically pure seeds of hybrid maize seeds COH(M)8 used in this experiment as source material were collected from the Department of Millets, Tamil Nadu Agricultural University, Coimbatore. The endophytic microorganisms *viz., B. bassiana, M. anisopliae and B. subtilis* were used for this study. The population dynamics or Colony Forming Unit (CFU)/ml solution of *B. bassiana, M. anisopliae* through Potato Dextrose Agar (PDA) medium *and B. subtilis* by Luria - Bertani (LB) medium was 2×10^{-8}.

Laboratory Experiment

The laboratory experiment was carried out at the Department of Seed Science and Technology, TNAU, Coimbatore during 2021-2022. The experiment was laid out with three replication in Completely Randomized Block Design (CRD). The maize seeds were surface sterilized with 0.5% sodium hypochlorite (NaOCl) solution for three minutes and washed with distilled water followed by priming with fungal endophytes *viz., B. bassiana* @ 5% (T_2), *M. anisopliae* @ 5% (T_3) and bacterial endophyte *B. subtilis* @ 8% (T_4) concentration along with hydropriming (water) (T_1) and Control (Nonprimed seeds) (T_0). Endophytic priming solution was prepared using double distilled water and seeds were soaked in priming solution for 12 hrs at the ratio of 1:1 and the temperature of 10-15^{o}C was maintained. The seed was removed from the solution after the priming (12 hrs) and rinsed with water and then dried back to its original moisture content. Dried seeds were used to assess the physiological and biochemical seed quality parameters and antioxidant activity.

Evaluation of physiological seed quality parameters

Speed of germination

The speed of germination was evaluated by the formula described by Maguire (1962).

$$\text{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + ... + \frac{X_n - X_{n-1}}{Y_n} \quad \ldots \quad \text{Eq. 1}$$

Where,

X_1- Number of seeds germinated at first count

X_2- Number of seeds germinated at second count

X_n- Number of seeds germinated on n^{th} day

Y_1- Number of days from sowing to first count

Y_2- Number of days from sowing to second count

Y_n- Number of days from sowing to n^{th} count

Mean germination time (MGT)

The number of seeds with a 2mm length of radicle emergence was measured and the mean germination time was calculated using the formula given by Ellis and Roberts (1980). The MGT was expressed in days.

Mean germination time (days) = ∑ nt / ∑ n ……… Eq. 2

n - Number of seeds germinated (2mm length of radicle emergence) at time t

t - Time form beginning of the radicle emergence

∑ n - Final radicle emergence

Germination (%)

The germination test was carried out with 50 seeds of eight replication as per International Seed Testing Association (2012). Germination percentage was calculated at end of the final count @ 7^{th} day after sowing, based on the number of normal seedlings.

Germination per cent =No. of normal seedlings/Total no. of seeds× 100 ….. Eq. 3

Seedling length (cm)

During the final count of the germination test, the root length and shoot length of ten randomly chosen normal seedlings were measured and expressed in cm.

Dry matter production (g 10 seedlings^{-1})

Seedlings selected for seedling length assessment were kept in a paper cover and dried in the shade for 24 hrs, followed by drying in a hot air oven at 80°C for 16 ± 1 hrs. After cooling in a desiccator, weight was taken and expressed in g 10 seedlings^{-1}.

Vigour index

The Vigour index was computed by the below formula given by Abdul Baki and Anderson (1973). The mean values are expressed in whole numbers.

Vigour index (I) = Germination (%) x Seedling length (cm) (Root length + Shoot length) ……… Eq. 4

Evaluation of biochemical seed quality parameters

α- amylase activity (mg maltose min^{-1})

α- amylase activity of primed and non-primed seeds was measured by the method of Paul *et al.* (1970) using the following formula and expressed as mg maltose min^{-1}.

α- amylase activity =(OD value/Volume of sample pipetted out) × 1000/500 …… Eq. 5

Dehydrogenase activity (OD value)

The dehydrogenase activity of the seed samples was determined by the method suggested by Kittock and Law (1968). The OD value obtained was reported as dehydrogenase activity.

Starch content (mg g^{-1})

The starch content was quantified for the primed and nonprimed seeds by the procedure described by Hodge and Hofreiter (1962) and was expressed as mg g^{-1} of the sample.

Glucose content = (OD value @ 630nm/Volume of sample) x Volume made upto 100 ml /1000 …. Eq. 6

Starch content = Glucose content × 100

Electrical conductivity (dSm^{-1})

The electrical conductivity of the seed leachate was measured by using a digital conductivity meter with a cell constant and the mean expressed as dSm^{-1} (Presley, 1958).

Evaluation of antioxidant activity

Catalase activity (μmol H_2O_2 min^{-1} g^{-1} protein)

Catalase activity of pre-germinated seeds of both primed and non-primed samples was assayed through the method of Aebi (1984). Enzyme activity was calculated as the concentration of hydrogen peroxide reduced and expressed as mmol H_2O_2 min^{-1} g^{-1} of seed.

Peroxidase activity (U mg^{-1}protein min^{-1})

Peroxidase activity of pre-germinated seed samples was determined by the protocol of Malik and Singh (1980). The peroxidase activity was expressed as U mg^{-1} protein min^{-1}.

Peroxidase activity = (Difference in OD value/10 min.) x (1000/500) x 60 …… Eq. 7

Superoxide dismutase (U mg^{-1}protein min^{-1})

Superoxide dismutase activity of bioprimed and control seeds was analyzed by the method prescribed by Beauchamp and Fridovich (1971).

Statistical analysis

The analysis of variance was conducded and a comparison was done by Duncan's Multiple Range Test (DMRT). The mean difference is significant at the P-values < 0.05. Statistical analysis was performed using the SPSS 16.0 software (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

Agriculture is India's most important source of economic and socio-political stability and employs the largest workforce in the country. Among the total geographical area, around 60% of the land is under cultivation, leading to soil deterioration due to the usage of more chemicals. In this context, the present study focused on the evaluation of beneficial microorganisms to enhance seed quality and boost the soil microbiome.

In the present study, endophytic seed priming with maize promotes the seed germination per cent and seedling growth. The significant difference (P<0.05) was registered in speed of germination, mean germination time and seedling length for all the endophytic treatments. The maximum speed of germination (7.12) was noted by *M. anisopliae* @ 5% (T_3) followed by *B. subtilis* @ 8% (T_4) (6.97), *B. bassiana* @ 5% (T_2) (6.88) and the control (T_0) recorded minimum value (6.45). Similarly, priming with *M. anisopliae* @ 5% (T_3) had a lower mean germination time (2.57) and the control (T_0) (2.98) was observed with a higher mean germination time. Primed seeds recorded superior values for speed of germination in most of the species. In a recent report, biopriming had reduced the mean germination time and increased the seedling vigour in paddy (Swain *et al.,* 2021). All the endophytic priming treatments in the present study recorded an increased shoot length (12.8% to 20.8%). Seed priming with *M. anisopliae* @ 5% (T_3) registered maximum shoot length (19.8 cm). Similarly, seed priming with *M. anisopliae* @ 5% (T_3) and *B. subtilus* @ 8% (T_4) recorded more root length of 23.7 cm and 23.4 cm, respectively, which were 15.04% and 13.59% over control. Endophytic fungi, enhance seedling growth by increasing nutrient assimilation and production of plant growth promoters such as gibberellin, indoles acetic acid (IAA), cytokinins and production of siderophores (Macuphe *et al*., 2021). Seed priming with three different endophytes had increased dry matter production (DMP) to the range from 13.59% to 15.22% over non-primed seeds. The highest dry matter production was noticed by *M. anisopliae* @ 5% (T_3) (1.415 g 10 seedlings^{-1}), which was on par with *B. subtilus* @ 8% (T_4) (1.401 g 10 seedlings^{-1}) while the control (T_0) (1.228 g 10 seedlings^{-1}) recorded the lesser value (Table 1). Seed priming with beneficial microbes can be associated with the elicitation of plant immunity, starting from the seedling stage itself (Song *et al.,* 2017). The increase in root and shoot weight by endophytes was due to the production of growth hormones like cytokinin, IAA and gibberellins thereby increasing cell division (Arkhipova *et al.,* 2005)

The significant difference ($P<0.05$) was registered in germination per cent for all the endophytic treatments. The highest germination per cent was observed in seed priming with *M. anisopliae* @ 5% (T_3) (96%), which was on par with *B. subtilus* @ 8% (T_4) (95%) and *B. bassiana* @ 5% (T_2) (95%) and the percentage of increase was 4.34%, 3.26% & 3.26% respectively over control. Increase in germination percentage might be due to the combined effect on the production of hydrolytic enzymes and growth hormones like IAA and gibberellins through endophytic seed treatments (Schulz and Boyle, 2005). This is in agreement with other studies showing that priming mainly improves germination as a result of the enhanced water uptake and more favourable water relations and also increases the production of hydrolytic enzymes in primed seeds (Lechowska *et al.,* 2019). Earlier report corroborates the findings of present study, e.g. Junges *et al*. (2016) observed that the germination and seedling growth of bean seeds primed with endophytes was significantly higher than control seeds. In another study, Russo *et al.* (2019) reported that the seed germination percentage was significantly increased in corn seeds treated with *B. bassiana*. In the present investigation, the vigour index values recorded by *M. anisopliae* @ 5% (T_3) (4176) was superior, when compared to control (T_0) (3404) with an increase of 22.68% (Fig. 1). Better seed germination with higher vigour of seedlings ultimately increases the quality of the seed. An increase in seed vigour might be due to the production of phytohormones like IAA, which was stated by Liao *et al*. (2017). Our findings were also in agreement with Gayathri *et al.* (2020). They recorded superior seed germination, seedling growth and seed vigour by seed priming with *M. anisopliae* in maize (*Zea mays* L.).

The difference in dehydrogenase activity, α- amylase activity and electrical conductivity of the maize seed leachate was significantly ($P<0.05$) influenced by seed priming with endophytes. Starch content was not significantly ($P>0.05$) influenced by the seed priming treatment. The activity of the dehydrogenase enzyme is a perfect and stable metabolic marker that evaluates the vigour status of the seed (Saxena *et al*., 1985). While

Table 1. Influence of endophytic seed priming treatments on physiological parameters in hybrid maize COH(M)8 seeds

Treatments	Speed of germination	Mean germination time (day)	Root length	Shoot length (cm)	Dry matter production (g 10 seedlings^{-1})
T_0	6.45±0.1 ^{c}	2.98±0.02 ^{c}	20.6±0.24 ^{d}	16.4±0.37 ^{c}	1.228±0.003 ^{d}
T_1	6.69±0.08 ^{bc}	2.82±0.07 ^{bc}	21.5±0.27 ^{c}	16.8±0.28 ^{c}	1.295±0.006 ^{c}
T_2	6.88±0.11 ^{ab}	2.64±0.02 ^{ab}	22.5±0.48 ^{b}	18.5±0.21^{b}	1.395±0.002 ^{b}
T_3	7.12±0.15 ^{a}	2.57±0.01 ^{a}	23.7±0.13 ^{a}	19.8±0.35 ^{a}	1.415±0.009 ^{a}
T_4	6.97±0.09 ^{ab}	2.65±0.04 ^{ab}	23.4±0.34 ^{a}	18.9±0.16 ^{b}	1.401±0.004 ^{ab}

(T_0 - Control, T_1 - Hydro priming, T_2 - Seed priming with *Beauveria bassiana* 5%, T_3 - Seed priming with *Metarhizium anisopliae* 5% & T_4 - Seed priming with *Bacillus subtilis* 8%)

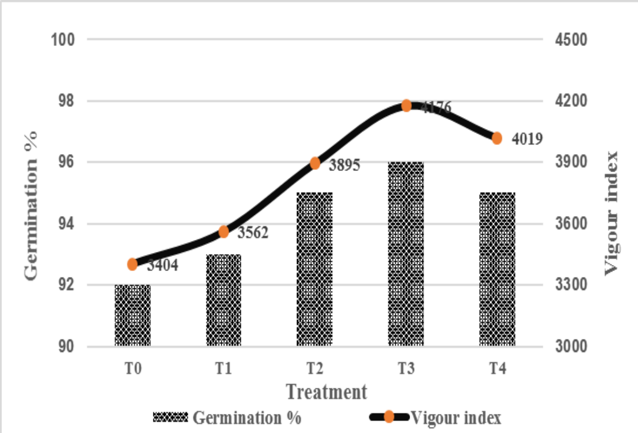

Fig. 1. *Effect of endophytic seed priming treatments on seed germination and seedling vigour in hybrid maize COH(M)8 seeds (T_0 - Control, T_1 - Hydro priming, T_2 - Seed priming with Beauveria bassiana 5%, T_3 - Seed priming with Metarhizium anisopliae 5% & T_4 - Seed priming with Bacillus subtilis 8%)*

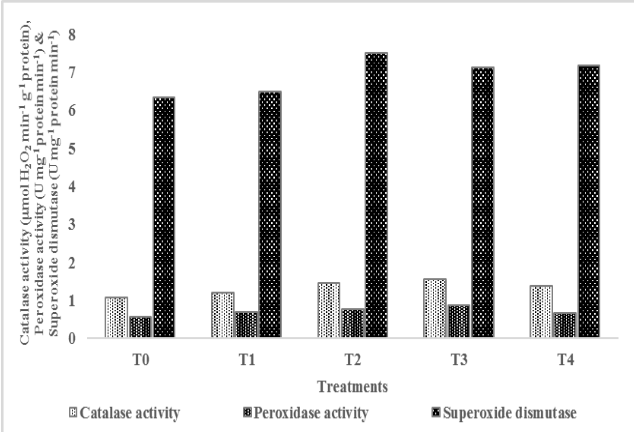

Fig. 2. *Effect of endophytic seed priming treatments on antioxidant activity in hybrid maize COH (M) 8 seeds (T_0 – Control, T_1 - Hydro priming, T_2 - Seed priming with Beauveria bassiana 5%, T_3 - Seed priming with Metarhizium anisopliae 5% & T_4 - Seed priming with Bacillus subtilis 8%)*

analyzing the dehydrogenase activity with different seed priming treatments, *M. anisopliae* @ 5% (T_3) (0.441 OD value) showed enhanced activity, which was on par with *B. subtilus* @ 8% (T_4) (0.434 OD value). However, the lowest dehydrogenase activity was noticed in control (T_0) (0.369 OD value). Kavitha *et al.* (2013) indicated that any seed treatment can enhance the dehydrogenase activity and α-amylase activity in sorghum. α- amylase is the key enzyme which plays an important role in hydrolyzing the seed starch reserve and supplying the sugars to the developing embryo. A quick resumption of α-amylase production immediately after imbibitions in primed seeds might be the reason for the rapid increase in α-amylase activity during germination (Nath, 1991). Results revealed that seed priming with *M. anisopliae* @ 5% (T_3) registered maximum α - amylase activity (2.06 mg maltose min^{-1}) followed by the *B. subtilus* @ 8% (T_4) (1.97 mg maltose min^{-1}) and *B. bassiana* @ 5% (T_2) (1.96 mg maltose min^{-1}) whereas control (T_0) recorded minimum α - amylase activity (1.16 mg maltose min^{-1}). In primed seeds, the activity of α-amylase tends to be increased which influences the hydrolysis of the starch (Afzal *et al.*, 2008). The same findings of present study was reported by Lee and Kim (2000) who observed that the α-amylase activity of rice seeds was increased through seed priming. The electrical conductivity of the seed leachate as a measure of membrane integrity is considered a good index for seed viability (Matthews and Bradnock, 1968) and vigour (Grabe, 1966). In the present investigation, the minimum electrical conductivity of maize seed leachate was recorded in seed priming with endophytes *viz., M. anisopliae* @ 5% (T_3) (0.196 dSm^{-1}), *B. subtilus* @ 8% (T_4) (0.208 dSm^{-1}) and *B. bassiana* @ 5% (T_2) (0.215 dSm^{-1}) and control (T_0) registered maximum (0.334 dSm^{-1}) (Table 2).

To determine the potential of priming with endophytes in eliciting an antioxidant response, the activity of antioxidant enzymes; catalase, peroxidase and superoxide dismutase were evaluated. Seed priming with *M. anisopliae* @ 5% (T_3) registered maximum value of the activity of antioxidant enzymes observed, followed by

Table 2. Influence of endophytic seed priming treatments on biochemical parameters in hybrid maize COH(M)8 seeds

Treatments	Dehydrogenase activity (OD value)	α - amylase activity (mg maltose min^{-1})	Starch content (mg/g)	Electrical conductivity of seed leachate (dSm^{-1})
T_0	0.369±0.008 ^{c}	1.16±0.038 ^{d}	59±0.50 ^{ns}	0.334±0.012 ^{b}
T_1	0.380±0.009 ^{c}	1.26±0.022 ^{c}	60±0.53 ^{ns}	0.322±0.005 ^{b}
T_2	0.422±0.002 ^{b}	1.96±0.010 ^{b}	58±0.55 ^{ns}	0.215±0.008 ^{a}
T_3	0.441±0.003 ^{a}	2.06±0.015 ^{a}	59±0.52 ^{ns}	0.196±0.004 ^{a}
T_4	0.434±0.005 ^{ab}	1.97±0.019 ^{b}	60±0.31 ^{ns}	0.208±0.009 ^{a}

(T_0 - Control, T_1 - Hydro priming, T_2 - Seed priming with *Beauveria bassiana* 5%, T_3 - Seed priming with *Metarhizium anisopliae* 5% & T_4 - Seed priming with *Bacillus subtilis* 8%)

seed priming with *B. bassiana* @ 5% (T_2). The difference in the activities of catalase, peroxidase and superoxide dismutase were significantly (P<0.05) influenced by endophytic seed priming treatments. Seed priming treatments with endophytes increased significantly catalase activity at 1.55 µmol H_2O_2 min^{-1} g^{-1} protein (*M. anisopliae* @ 5% (T_3)) whereas control (T_0) recorded the lowest activity of 1.08 µmol H_2O_2 min^{-1} g^{-1} protein. These results are in harmony with earlier studies, where seed priming with endophytic bacteria resulted in increased catalase activity of rice (Chakraborty *et al*., 2011; Swain *et al.,* 2021). Similarly, for peroxidase activity

M. anisopliae @ 5% (T_3) observed maximum activity (0.87 U mg^{-1}protein min^{-1}) and seed priming with *B. subtilus* @ 8% (T_4) and hydro priming (T_1) had similar effects on peroxidase activity. While analyzing the superoxide dismutase of various treatments, *B. bassiana* @ 5% (T_2) showed higher activity (7.51 U mg^{-1}protein min^{-1}) than other treatments (Fig. 2). Increased synthesis of enzymatic antioxidants results in the reduction of lipid peroxidation rate. Radhakrishnan *et al.* (2013) reported that soybean seeds treated with endophytic fungi enhance catalase, peroxidase and superoxide dismutase activity to counteract lipid peroxidation thereby reducing free radical. The same findings were also reported by Li *et al.* (2019), who recorded increased activities of superoxide dismutase and catalase when the seeds were treated with endophytes in maize.

Conclusion

The present study demonstrated the beneficial effects of endophytes when used for seed priming treatments to enhance physiological, biochemical properties and antioxidant activities of hybrid maize COH(M)8 seeds. Among different endophytes (*B. bassiana, M. anisopliae and B. subtilis*), *M. anisopliae* @ 5% (T_3) was found to be superior for hybrid maize seeds. It is concluded that seed priming with *M. anisopliae* @ 5% could be used as an effective seed priming treatment in maize (*Zea mays* L.) for seed quality improvement.

Conflict of interest

The authors declare that they have no conflict of interest.

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