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Short Communication

Unveiling the Potential of Fungal Endophytes for Abiotic Stress Mitigation in Wheat

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Fungal endophytes are microorganisms that reside within the tissues of plants without causing apparent harm to the host. They often show symbiotic relationships with the plants by providing various benefits such as improved nutrient uptake, growth promotion, and enhanced resistance to biotic and abiotic stresses. Numerous studies have consistently demonstrated the ability of fungal endophytes in imparting abiotic stress tolerance in multiple crops (Manasa *et al.,* 2020; Ali *et al.,* 2022, Bouzouina *et al.,* 2021, Verma *et al.,* 2022). In the context of wheat (*Triticum aestivum* L.), which serves as a major global food source, frequent exposure to abiotic stresses severely affects productivity and grain quality and harnessing fungal endophytes offers a sustainable, eco-friendly, and cost-effective approach for improving stress tolerance without compromising yield and can significantly contribute to the development of climate-resilient wheat varieties. However, research on fungal endophyte associations in wheat remains scarce (Fan and Shi, 2024; Dargiri *et al.,* 2025) compared to other crops like rice and maize. Therefore, present study aims to bridge this gap by evaluating the potential of two stress-tolerant fungal endophytes in improving abiotic stress tolerance in the wheat genotype HD2009. Based on the previous findings in rice, two stress tolerant fungal endophytes *Fusarium incarnatum* -K23 (PMB1) from Kargil and *Fusarium equiseti* -SF5 (PMB2) from coastal sand dunes of Tamil Nadu (Manasa *et al.,* 2020; Pallavi *et al.,* 2024) were procured from UAS, GKVK, Bengaluru and were used in the study at Indian Council of Agricultural Research-Indian Institute of Wheat and Barley Research (ICAR-IIWBR, Karnal). The primary objective of the study was to assess the effects of these endophytic fungi for enhancing abiotic stress tolerance in wheat. The ten days old fungal cultures were used to prepare spore suspension of 2 x 10^6 spores/ml concentration. Two days old uniformly germinated HD2009 wheat genotype was incubated in fungal suspension for three hours, whereas seeds incubated in sterile water served as a control (Sangamesh *et al.,* 2017). The seed treatment was done at UAS, GKVK, Bengaluru and then sent to ICAR-IIWBR. Further, treated seedlings obtained were transplanted with three replications into 4″ plastic pots filled with a mixture of soil, sand, and peat in a 2:1:1 (w/v) ratio. Plants were grown upto 45 days under controlled laboratory condition at a temperature of 22°C and relative humidity of 65±10% followed by a photoperiod of 16 hours of light and 8 hours of dark. After 45 days, the uniform leaf samples were plucked from the seedlings and excised leaf sample assay was carried out with four replications of leaf samples by dipping in 20% polyethylene glycol (PEG-6000) solution, 250mM NaCl and by exposing leaves in petri plates at 40^0C for inducing drought, salt and heat stress respectively (Fig. 1) (Kumar *et al.,* 2020). After stress induction, clear variation in greenness in control and endophytes treated leaves were seen in petriplates. Further, the variation in greenness of leaf tissues under different treatments was estimated by quantifying total chlorophyll content using colorimetric method as described by Hiscox and Israelstam (1979). It was observed that, there was less reduction in total chlorophyll content in PMB1- K23 and PMB2 – SF5 stress leaf tissues as compared to control stress under drought, heat and salt stress (Fig. 2) conditions and are found to be statistically significant. The experimental data were analyzed using the SAS statistical software, PROC GLM, SAS version 9.3 (SAS Institute Inc. Cary, NC, USA) at different statistical significance level. The mean values with different alphabets are found to be statistically significant. These initial results confirmed the positive effect of endophytes in imparting abiotic stress tolerance.

Fig. 1: Effect of different stresses on control and endophytes treated (PMB1- K23 and PMB2- SF 5) leaves of wheat (HD 2009)

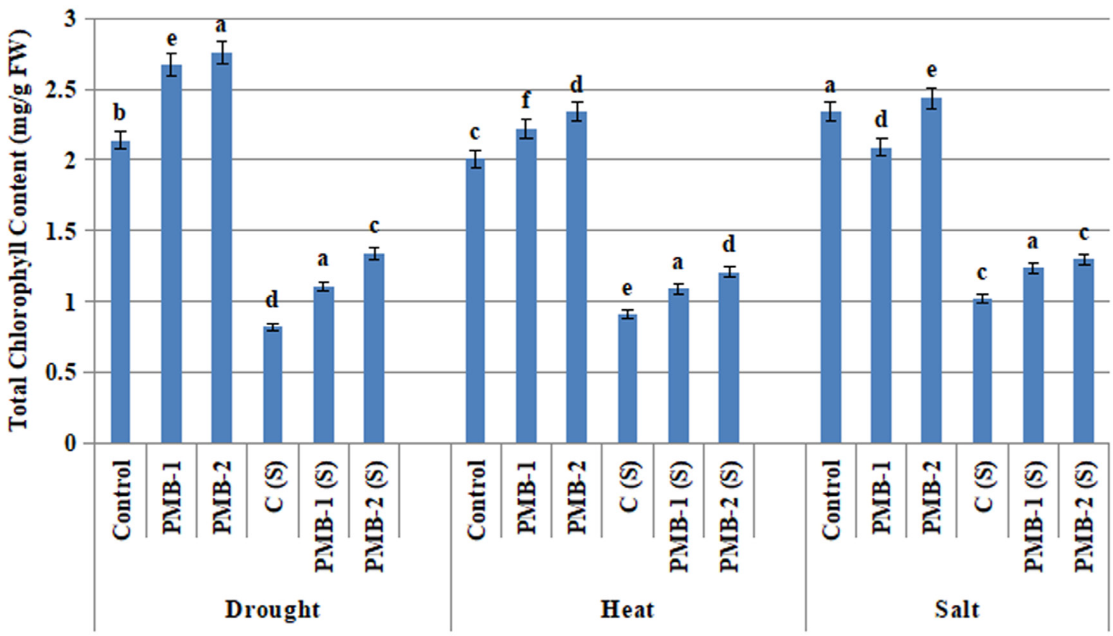

Fig. 2: Variation in total chlorophyll content of control and endophytes treated (PMB1- K23 and PMB2- SF5) wheat plants under drought, heat and salt stress conditions.

After assuring the positive effect of endophytes in inducing abiotic stress tolerance in excised leaf disc assay, pot experiment was conducted wherein, plants of HD 2009 in pots were subjected to drought stress by withholding water for 15 days after anthesis in three replications. After stress imposition, the physiological traits like Assimilation rate, Stomatal conductance (gs), Water use efficiency (WUE) were measured using Infra red gas analyser (IRGA, CIRUS 3, Nutech), Chlorophyll content Index (CCI) using Chlorophyll meter (SPAD-Minolta -502 Plus),Variable fluorescence/maximal fluorescence (Fv/Fm) using Fluorescence meter (Optisciences), Nitrogen balance Index (NBI) using Chlorophyll polyphenol meter (Optisciences), Electrical conductivity (EC) using EC meter (Eutech), Osmotic potential (OP) using Vapro osmometer as per the manufacturers guidelines and Relative water content (RWC) as per Barrs & Weatherley, 1962. The drought stress-imposed endophytes treated seedlings showed significant morpho-physiologial variation as compared to control and they were able to withstand high chlorophyll retention and better physiological adaptations as compared to stress control plants (Fig. 3 & Table 1). It was found that, the assimilation rate (A) was higher in PMB2 & PMB1 as compared to stress control plants. Similar trends were observed for stomatal conductance (gs), water use efficiency (WUE) chlorophyll content index (CCI), chlorophyll fluorescence (Fv/Fm), Nitrogen balance index(NBI), and Relative Water Content (RWC) which are positive regulators of stress tolerance. The Electrical conductivity (EC%) and osmotic potential (OP) are found to be high in control stress plants followed by PMB1 and PMB2 stress plants.

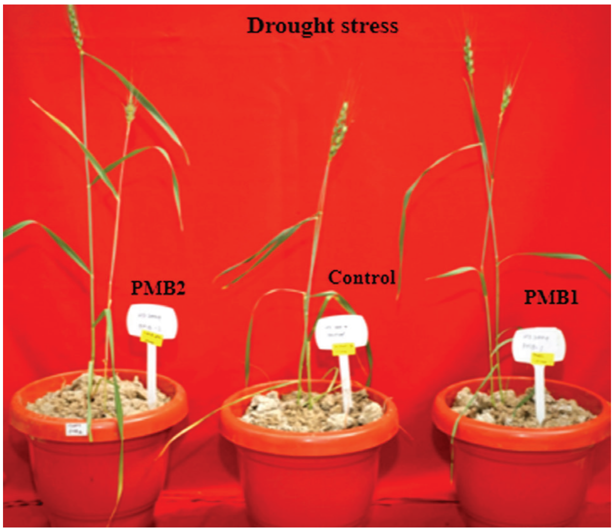

Fig. 3: Variation in phenotype of control and endophytes treated wheat plants (HD 2009) under drought stress

Table 1: Various physiological traits (Mean values) under control and drought stress in wheat genotype HD 2009 treated with endophytic fungi (PMB1 and PMB2)

Treatments	A (µmol CO_2 $m^{-2}.S^{-1}$)	gs (mmol H_20 m^{-2} S^{-1})	WUE (mmol CO_2 $mol^{-1}H_20$)	CCI	Fv/Fm	NBI	EC (%)	RWC (%)	OP (-MPa)
Control	18.7^{c}	300^{c}	4.3^{ba}	42.07^{a}	0.78^{a}	34.2^{c}	20.6^{ba}	87.9^{ba}	0.92^{b}
Control Stress	15.4^{c}	270^{d}	3.1^{b}	28.23^{d}	0.69^{a}	28.3^{dc}	25.0^{a}	48.9^{c}	1.97^{a}
PMB1 Control	23.8^{a}	362^{a}	5.1^{a}	42.37^{a}	0.79^{a}	36.1^{bc}	20.4^{ba}	87.8^{ba}	1.11^{b}
PMB1 Stress	19.3^{b}	309^{c}	4.4^{ba}	34.27^{c}	0.76^{a}	32.4^{dc}	23.9^{a}	56.5^{c}	2.03^{a}
PMB2 Control	24.5^{a}	340^{b}	5.3^{a}	43.63^{a}	0.79^{a}	46.2^{a}	19.2^{b}	90.5^{a}	1.05^{b}
PMB2 Stress	20.1^{b}	305^{c}	4.6^{a}	38.93^{b}	0.78^{a}	42.4^{ba}	21^{ba}	73.0^{b}	1.98^{a}

Assimilation rate, gs –Stomatal conductance, WUE- Water use efficiency, CCI-Chlorophyll content Index, Fv/Fm- Variable fluorescence/maximal fluorescence, NBI-Nitrogen balance Index, EC- Electrical conductivity, RWC- Relative water content, OP – Osmotic potential

In summary, the findings from both the excised leaf disc assay and pot experiments clearly demonstrate that the selected fungal endophytes possess strong potential to successfully colonize wheat tissues and enhance tolerance to abiotic stresses. Their inoculation not only improved the plants resilience under stress conditions but also positively influenced several physiological parameters–such as chlorophyll content, relative water content, and overall growth performance–even under non-stress (control) conditions compared to untreated plants. Similar beneficial effects of endophytic inoculation have been reported in other cereals such as rice and maize where endophytes improved drought tolerance, nutrient uptake, and antioxidant activity through modulation of hormonal balance, enhanced osmolyte accumulation, and upregulation of stress-responsive genes. Recent work in wheat by Dargiri *et al.* (2025) reported that *Penicillium chrysogenum* CM022 enhances wheat tolerance to salinity (150 mM NaCl) by improving germination, growth, and photosynthetic pigment retention while reducing oxidative damage. The endophyte increased osmolyte accumulation and antioxidant enzyme activity, highlighting its potential as a biological agent for sustainable salinity stress management in wheat. Fan and Shi (2024) reviewed the diversity and biochemical potential of endophytic fungi associated with the four major staple crops wheat, rice, maize, and potato where isolation of various metabolites from different fungal endophytes includes bioactive molecules that have the potential applications in crop protection and stress tolerance mechanism. These parallels suggest that the observed improvements in wheat may involve comparable physiological and molecular mechanisms such as the regulation of reactive oxygen species (ROS) scavenging enzymes, increased production of indole acetic acid (IAA), and activation of stress-related signalling pathways. However, while the controlled environment studies provide strong experimental evidence of their effectiveness, field-based validation with more genotypes remains essential to confirm the consistency, magnitude, and stability of these effects across different agroecological conditions. Moreover, a deeper understanding of the mechanisms of endophyte colonization, persistence, and vertical transmission to subsequent generations will be crucial for their sustainable application in wheat improvement programs. Integrating such beneficial fungal endophytes into crop management or breeding strategies could represent a promising, eco-friendly, and cost-effective approach towards developing climate-resilient wheat cultivars suited to future environmental challenges.

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Author contributions

MHM: Conceptualization of experiment and edited the manuscript, ZW: wrote the main manuscript and data recording, ARR, PAS, NS: Endophyte treatment and other logistics at UAS, GKVK, PR: Figures and table, NKN: Conceptualization of experiment and edited the manuscript, OP, RT: Overall guidance and support. All authors read and approved the manuscript.

Declaration of Competing Interest

The authors declare that there is no competing interest.

Ethical Approval

The article doesn't contain any study involving ethical approval.

Generative AI or AI/Assisted Technologies use in Manuscript Preparation

No

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